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FECUNDATION IN PLANTS

BY

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PREFACE.

This volume presents the subject of fecundation in the vegetable kingdom by the discussion of concrete cases, selecting from the great groups of plants certain typical representatives in which the sexual process seems to have been most thoroughly investigated. In the introductory chapter I have discussed typical processes of nuclear division and cell-formation, especially in spore mother-cells, together with a few topics dealing with certain phenomena of the cell and the significance of sexuality. This is considered necessary to a better understanding of sexual reproduction, for problems of sexuality, like problems of evolution, have in late years become reduced to problems of the cell, and, since the nucleus plays by far the most important part in fecundation, I am tempted to say to problems of the nucleus.

The processes leading to the development and differentiation of the gametes have been regarded as of prime importance, and they have therefore received emphasis. Whenever the subsequent history of the fecundated egg has been followed to any extent this has been done, as in the *Ascomycetes* and *Florideæ*, to show the relation between the real sexual process and the vegetative fusion of nuclei which has been confused with the sexual act, and, as in the Desmids, for the sake of pointing out certain nuclear phenomena that take place during the germination of the zygote with similar phenomena just preceding the sexual act in the Diatoms. Processes which are purely morphological are assumed or dealt with very briefly.

In grouping the representative types into the several chapters I have had in mind no particular theory of the evolution of sexuality, but merely the idea of the evolution of the plant kingdom and the corresponding differentiation of the sexual organs and cells accompanying this evolution in the groups of plants themselves.

The chapters dealing with the lower plants in which the development of the gametes is not known from a modern cytological standpoint, and in which the behavior of the sexual nuclei in the fusion of the gametes has not been followed—have been made as brief as possible. For a similar reason the mosses and liverworts have been omitted entirely.

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No attempt has been made to discuss the numerous theories bearing upon the subject. Whenever theoretical matters are touched upon the object has been chiefly to suggest probable lines of investigation. I have not hesitated, however, to express my own opinion in all cases in which my special field of study has given me a first-hand knowledge of the subject-matter.

To designate the sexual process which consists in the fusion of sexually differentiated cells, or gametes, and especially the fusion of their nuclei, the term *fecundation* has been used instead of *fertilization*—*fecundation* being the equivalent of the German *Befruchtung* and the French *fécondation*.

It has been necessary, of course, to copy numerous figures from the papers of other investigators, but in every case due credit is given.

In the citation of literature in the text the author is referred to by the year in which his work was published. No attempt has been made to give a complete bibliography, and no doubt many valuable references have been omitted.

The author is indebted to Professors W. Belajeff, H. O. Juel, F. Oltmanns, S. Ikeno, and to Dr. H. Klebahn, Dr. A. H. Trow, Dr. H. Wager, Dr. S. Hirase, and Dr. V. H. Blackman, for reprints of their papers, from many of which illustrations have been borrowed, and especially to Professor R. A. Harper for helpful suggestions.

DAVID M. MOTTIER.

INDIANA UNIVERSITY, *August, 1902*.

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FECUNDATION IN PLANTS.

CHAPTER I.—INTRODUCTION.

The processes of nuclear division and cell-formation are so closely associated with sexual cells and their development that an adequate understanding of these cells is impossible without a definite and thorough knowledge of the processes involved in their development. Our interpretations of the significance of the sexual process and the phenomena of heredity in all organisms will be more lasting and helpful as scientific knowledge if these interpretations or doctrines are based upon a well-connected phylogenetic series of the most fundamental facts. Perhaps no other field of research has been more helpful during the past quarter of a century in enabling the biologist to gain a deeper and more far-reaching knowledge of the physical basis of heredity than the study of mitosis, especially in reproductive cells. The division of the nucleus naturally suggests the division of the cell, or the process by which new cells are formed from a mother-cell, and the study of cell-formation in very recent years, especially among the lower plants, has not only wrought almost a revolution in our knowledge of the processes here involved, but has also furnished new criteria for determining relationships and probable lines of descent.

It is deemed necessary, therefore, to introduce the subject of sexual reproduction in plants by a brief presentation of the typical processes of nuclear and cell-division in both the lower and higher forms. In doing so these processes will be described in a few of those forms which have been subjected to a critical study by means of the most improved methods and instruments. The processes described will be confined largely, though not exclusively, to spore mother-cells.

The division of the nucleus and of the cell presents generally three processes, the development of the karyokinetic spindle, the behavior of the chromatin, and the formation of the cell-plate or new plasma membrane. This division is made merely for the sake of convenience, as it is not implied that three distinct or separate processes are necessarily involved, although the development of the plasma membrane in many cases has apparently little or no connection with the

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division of the nucleus. The first two of these processes will be discussed under *nuclear division*, while the third will be dealt with in connection with *cell-formation*.

NUCLEAR DIVISION.

KARYOKINESIS IN CELLS OF THE LOWER PLANTS IN WHICH CENTROSOMES AND CENTROSPHERES ARE DEVELOPED.

At present there are recognized two types of development of the karyokinetic spindle. In one the spindle arises through the instrumentality of individualized dynamic centers or centrospheres, as in certain Thallophyta and Liverworts; in the other, it is developed wholly independently and in the absence of any such centers, as, for example, in the higher plants. We speak of types of spindle development in this connection also for the sake of convenience, since centrospheres have not been found in connection with the development of the spindle in all Thallophytes; but the author does maintain that centrospheres have not been demonstrated to occur in any plant above the Bryophytes, and that in the Angiosperms such structures do not in all probability exist.

As illustrating the development of the spindle in which centrospheres are present, the tetraspore mother-cell in *Dictyota dichotoma* will be selected from the algæ and the mother-cell of the ascus in *Erysiphe* from the fungi.

It is not considered necessary, nor conducive to any better understanding of the facts presented here, to enter into any lengthy discussion concerning the structure of the firmer framework of the cytoplasm. The consensus of opinion now is that the firmer substance of cytoplasm consists of either a reticulum of fibrillæ or of an alveolar or foam structure (Waben of German literature) and that, in many cells, these two structures intergrade into one another.

DICTYOTA.

The cytoplasm of the tetraspore mother-cell of *Dictyota dichotoma* during the preparation for nuclear division presents two well-defined portions, the kinoplasm, which is always associated with the nucleus and plays the most important rôle in the karyokinetic process, and the remaining alveolar portion. Numerous chloroplasts are also present.

The first indication of mitosis is the appearance, on opposite sides of the nucleus, of two large sharply defined asters of kinoplasmic fibers radiating from a rod-shaped body, which is often slightly bent, lying either close to the nuclear membrane or at some little distance from it (Fig. 1, A). The rod-shaped body is the *centrosome*, which

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together with the kinoplasmic radiations constitutes the *centrosphere*. The planes of the longitudinal axes of the centrosomes may be parallel or form various angles with each other. In Fig. 1, B, the centrosome at the upper side of the nucleus is seen from the side, the lower from

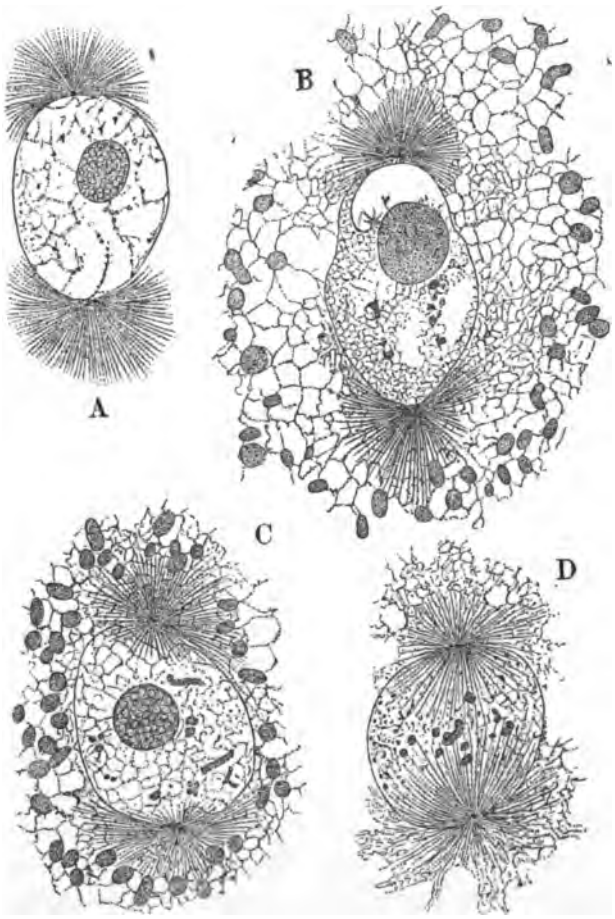

FIG. 1.—First mitosis in tetraspore mother-cell of *Dictyota dichotoma*.

A, B, early prophase; the well-developed centrospheres are on diametrically opposite sides of nuclei.
C, the kinoplasmic fibers have begun to enter the nucleus to form the spindle and the chromosomes are being differentiated.
D, numerous spindle fibers have entered the nucleus, and the chromosomes are collected in the equatorial region.

the end. Viewed from the pole, the centrosome is always rod-shaped. The kinoplasmic fibers radiate in all directions into the cytoplasm where they pass over into the framework of the same. On the side next the nucleus they may run parallel with its wall for some dis-

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tance. Near the nucleus the cytoplasm is more granular, with smaller meshes. It is more nearly a thread-like net-work than alveolar in structure, and appears with differential staining as kinoplasm. This very fine granular thread-work often extends in among the radiations of the centrosphere.

The resting nucleus shows a large vacuolated nucleolus and a fine linin-reticulum with rather large meshes, upon which are arranged small and nearly uniform granules, all of which do not react as chromatin. With the advance of karyokinesis, the chromatin begins to collect into larger and somewhat irregular masses that finally become the chromosomes. There is not developed, as in vegetative cells of this plant, a regular and uniform chromatin spirem or ribbon. The nucleolus becomes more vacuolated and soon disappears. The nuclear cavity presents a more granular appearance, the granules staining more densely.

The kinoplasmic fibers now penetrate the membrane of the nucleus and enter its cavity, while at the same time the polar radiations seem to diminish in number (Fig. 1, C). On entering the cavity some of the fibers proceed in advance of the others. Some pass straight toward the center of the nucleus, while others diverge toward the sides. As these fibers approach from opposite sides of the nucleus, they tend to collect the chromosomes into an irregular mass in the equatorial region, where they finally form the nuclear plate (Fig. 1, D). Certain of these fibers coming from opposite sides seem to unite at their ends to form the continuous spindle fibers which extend from pole to pole; others fasten themselves to the chromosomes, and still others diverge toward the nuclear membrane in the equatorial region (Fig. 2, E). In the mature spindle, therefore, the fibers present the following orientation: those radiating from the poles, the continuous spindle fibers extending uninterruptedly from pole to pole, those running from the poles to the chromosomes, and the fibers which diverge from the poles toward the equatorial region and end in the cytoplasm (Fig. 2, F).

The nuclear membrane in the tetraspore mother-cell of *Dictyota* disappears very gradually during the process of karyokinesis, often persisting at the sides when the spindle is mature (Fig. 2, F). It begins to disappear at the poles as soon as the fibers enter the nuclear cavity, and by the time the anaphase is reached no part of the membrane can be distinctly seen. Thus the spindle, with the exception of the polar radiations, lies within the nuclear cavity, its fibers, however, being largely of cytoplasmic origin. To what extent any nuclear substance contributes to the formation of the spindle is difficult to determine.

On the disappearance of the nucleolus, numerous granules appear in

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the nucleus, which stain deeply, closely resembling the chromatin granules. In the meantime the chromosomes increase in size, and it seems reasonable to suppose that the nucleolar substance contributes materially to their growth. The development of the nucleolus in the daughter nucleus and its behavior during the following, or second mitosis, seem to strengthen this theory. The chromosomes, when

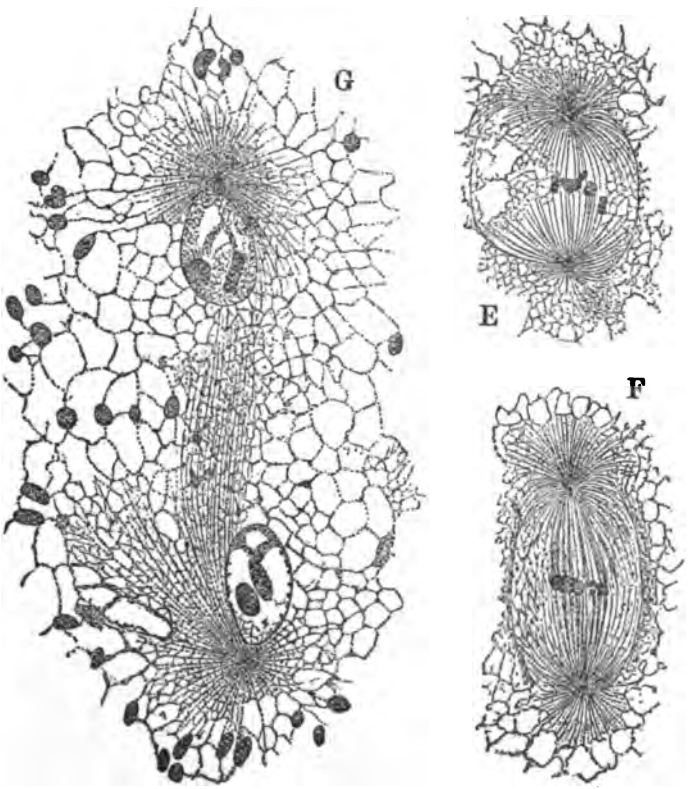

FIG. 2.—Spindle and telophase of first mitosis in the tetraspore mother-cell of *Dictyota dichotoma*.

E, spindle nearly mature; nuclear membrane has disappeared at poles.

F, mature spindle; the small lumpy chromosomes are regularly arranged in equatorial plate; nuclear membrane persists at sides.

G, daughter nuclei still connected by strand of connecting fibers; at poles of each nucleus is a well-developed centrosphere.

arranged in the equatorial plate, appear, especially when crowded together—a phenomenon of frequent occurrence—as rounded lumps (Fig. 2, E, F). A careful study in favorable cases shows clearly that each chromosome is either in the shape of a ring, or so contracted as to leave scarcely any central space, such, for example, as occurs in some higher plants (*Podophyllum*, *Helleborus*). In such cases each

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segment or daughter chromosome forms one-half of the ring, or each may be in the form of a short, thick U (Fig. 2, F). Sixteen chromosomes, the reduced number, are present in the first mitosis.

While on the way to the poles the daughter chromosomes sometimes fuse with one another to form large masses.[1] This is especially so in the second mitosis.

In the construction of the daughter nuclei, one or more larger masses of chromatin are formed by the chromosomes; a nucleolus appears near the chromatin mass or masses, and a nuclear membrane is laid down (Fig. 2, G). The membrane is unquestionably formed through the agency of the kinoplasmic fibers. The centrosomes increase in size, and the polar radiations are more distinct than in the spindle stage. The connecting fibers usually persist until the nuclear membrane is present, but a little later they disappear entirely. The chromatin mass, gradually becoming less dense, soon disintegrates, and each daughter nucleus passes into the resting condition (Fig. 2, G).

From the preceding it will be seen that each daughter nucleus is provided with one centrosome, but in the first mitosis the centrosomes could not be made out until they were on opposite sides of the nucleus and provided with radiations. The question naturally arises: Does the centrosome divide to give rise to the two daughter centrosomes?

Swingle ('97), who has traced the persistence of the centrosome through several successive generations of vegetative cells in *Stypocaulon*, one of the *Phæophyceæ*, found that a division of the centrosome takes place, and Strasburger ('97) arrives at the same conclusion as regards *Fucus*. This is the generally accepted view.

We shall trace the early development of the spindle in the second mitosis in the tetraspore mother-cell in order to see what evidence is furnished by *Dictyota* toward the solution of this problem.

During the reconstruction of the daughter nucleus (Fig. 3, H) two rod-shaped centrosomes, each with its radiations, were observed close together, and in such a position as to form a wide V, giving the impression that a longitudinal division of the single centrosome had taken place. The manner in which a cluster of radiations is attached to each daughter centrosome seems to lend weight to this conclusion.

The daughter centrosomes now separate, moving along the nuclear membrane, but they do not, as in the first mitosis, traverse an angular distance of 180° before the formation of the spindle begins (Fig. 3, I, K). The development of the spindle is the same as in the first mitosis, as Fig. 3, I, J, K, L, will clearly show.

In other brown algæ, so far as known (Swingle '97, Strasburger '97),

[1] This massing of the chromosomes may not occur in all cases.

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the development of the karyokinetic spindle in both vegetative and reproductive cells agrees essentially with that described for *Dictyota*.

In the diatoms the development of the spindle as described by Lauterborn ('96) is singular and without parallel in the plant kingdom. According to this author, the spindle develops directly from the centrosome by a division of the same or by budding. We shall refer to this phenomenon beyond in the section dealing especially with the centrosome. In the red algæ the development of the karyokinetic figure is known somewhat in detail only in *Corallina officinalis*. In this plant, Davis ('98) finds that the spindle arises through the agency

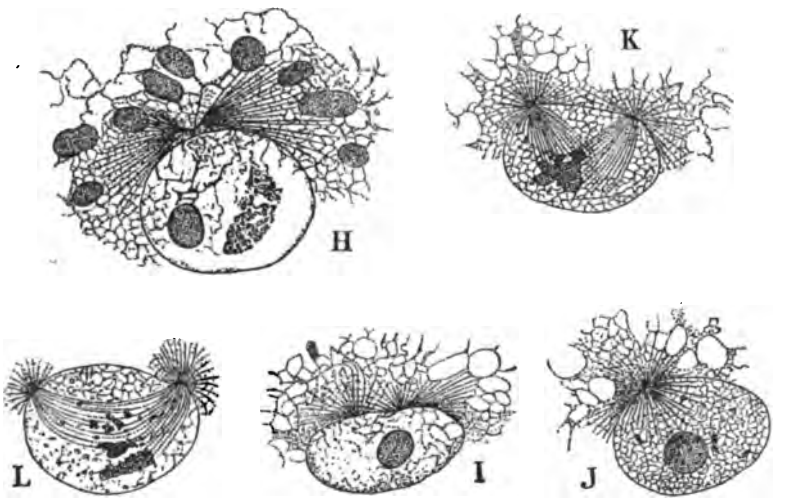

FIG. 3.—Second mitosis in tetraspore mother-cell of *Dictyota*.
H–K, prophase, showing origin of spindle. L, a nearly mature spindle.

of centrospheres which undergo a great change in size during mitosis. The persistence of these bodies was not followed from one cell generation to the next. The paucity of our knowledge of nuclear division in the red algæ precludes any further mention of the subject in this group of plants. So far as is known to the author, no centrospheres or centrosomes have been authentically observed in the green algæ.

ERYSIPHE COMMUNIS.

For the fungi, the most accurate and complete account of karyokinesis is to be found in the classical work of Harper ('97) on certain *Ascomycetes*. As an illustration of the process in this group of fungi, which is probably best known cytologically, a brief account of mitosis will be given as described by Harper in the ascus of *Erysiphe communis*.

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The ascus of this species offers unusually favorable material for the study of mitosis on account of the clearness with which all details are brought out, and because the three successive nuclear divisions follow each other rapidly, making it possible to trace with unmistakable clearness the persistence of the centrosome from one nuclear generation to the other. Since the spindles lie in different planes, it is possible also to observe, side by side, the same stages at different angles in the same field of the microscope. The following refers especially to the second mitosis in the ascus.

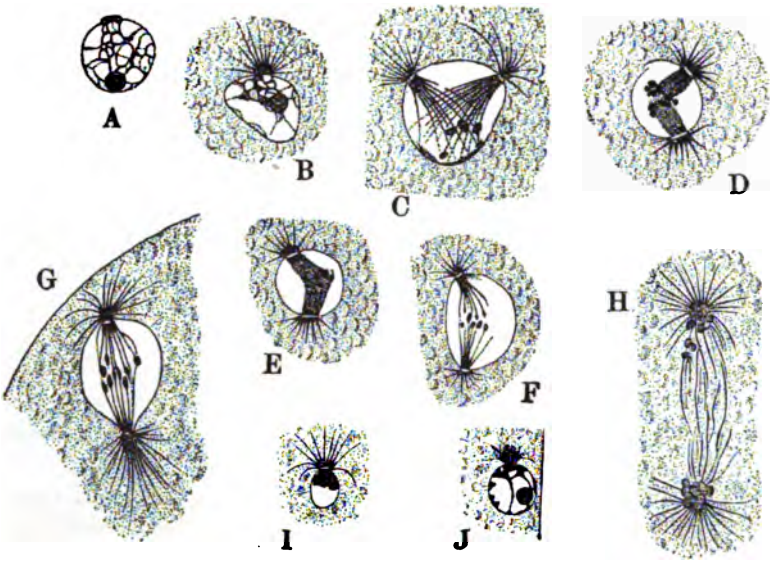

FIG. 4.—Mitosis in ascus of *Erysiphe communis*.—(After Harper.)

A, nucleus in resting stage of second nuclear generation in ascus, the flattened or disk-shaped centrosome closely applied to nuclear membrane.

B, early prophase; the kinoplasmic radiations have been developed about the centrosome.

C, D, E, F, successive steps in development of spindle.

G, mature spindle, the nuclear membrane still persists at sides.

H, end of anaphase; connecting fibers extend between the daughter nuclei, which are not yet provided with a nuclear membrane.

I, daughter nucleus provided with membrane, kinoplasmic radiations present.

J, later stage in which the polar radiations have disappeared.

Between the successive nuclear divisions in the ascus, the chromatin of the daughter nuclei does not assume the complete resting condition. It consists (Fig. 4, A) of an irregular net with the angles of the meshes somewhat thickened. Generally the net lies tolerably free in the nuclear cavity, and a very distinct nucleolus is present. The centrosphere appears as a flattened disk closely applied to the nuclear membrane, giving the impression as if the two were grown together (Fig. 4, A). The chromatin net appears also attached at this place

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and frequently forms a dense mass. These phenomena indicate clearly that chromatin and centrosphere are in direct communication through the nuclear membrane. The first step in the division is characterized by the appearance of a well-developed aster or system of radiations about the centrosome. It seems very probable here that the radiations grow out into the cytoplasm from the centrosome as a center. In the development of the radiations the nucleus probably coöperates. At this stage the chromatin is contracted into a dense net toward the centrosphere and appears in close connection with it. From the chromatin mass several fine achromatic threads extend toward the nuclear membrane (Fig. 4, B).

In the next stage observed, the two poles of the spindle have been formed, which lie some distance apart on the nuclear membrane (Fig. 4, C). The polar radiations are well developed, and from each centrosome a cone of spindle fibers extends into the nuclear cavity. The diverging fibers seem to be inserted in the nuclear membrane at points opposite the centrosome. As in *Dictyota* the two systems of fibers cross each other at nearly right angles without in any way uniting. Whether the two centrospheres arose by a division of the primary centrosphere cannot be stated with absolute certainty, since the intermediate stages between B and C, Fig. 4, were not observed, yet from what is known in *Stypocaulon* and in *Dictyota*, it seems reasonable to suppose that the centrosphere may undergo a division in *Erysiphe* also.

The chromatin, at this stage, seems to be reduced in mass to that which will appear in the nuclear plate. It lies distributed in irregular lumps among the fibers opposite the two poles. The nucleolus has now disappeared, or, in some cases, it may remain in the form of a weakly staining residue. The spindle fibers within the nucleus become attached to the chromosomes and then contract strongly, bringing the chromosomes into the center of the nuclear cavity (Fig. 4, C, D, E, F). Some of the fibers of the bent spindle appear, at this stage, to extend uninterruptedly from pole to pole. The continuous fibers are, in all probability, formed by the union of those which are not attached to the chromosomes.

The polar radiations now undergo a marked change, becoming shorter and thicker, as if drawn in toward the poles. The majority of the radiations diverge only slightly. They are contracted into bundles or brush-like collections, which stand perpendicular to the surface of the nucleus. Some of these radiations, however, diverge somewhat from the central group, but all the polar radiations are not centered upon a single point. The pole of the spindle is exactly as broad as the base of

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the central group of polar radiations, and, as will be seen from Fig. 4, E, F, G, the impression is that the polar radiations and the spindle contain the same number of fibers, which are continued uninterruptedly through the poles. But the continuity of the fibers is sharply interrupted by an achromatic plane at the nuclear membrane, through which the deeply staining (violet, by the Flemming triple stain) fibers pass from nucleus to cytoplasm. Whether the spindle fibers actually end at the nuclear membrane, or whether their substance only stains less densely there, was not determined. However, the phenomenon leaves the impression that the central body consists merely of the bases of the polar radiations closely crowded together. If the centrosome is an individual organ here, it seems that it must consist of a very thin, flattened disk, equal in breadth to the blunt end of the spindle.

The poles of the spindle now separate farther from each other, whereby the spindle becomes straight. The individual chromosomes, eight in number, which are arranged in the equatorial plate, are sharply defined, and the nucleus has become somewhat elongated (Fig. 4, G). The polar radiations have again become fine elongated fibers, forming regular systems of sun-like radiations.

As soon as the daughter chromosomes have reached the poles of the spindle the nuclear membrane disappears (Fig. 4, H). The fibers of the central spindle become now less sharply defined and broken in different places. Their number is also gradually diminished, their substance soon being indistinguishable from the immediately surrounding cytoplasm. The polar radiations, however, form at this stage a more regular and sharply defined aster, owing to the outer rays bending somewhat backward round the chromosomes (Fig. 4, H). The latter form a dense mass in which the individual elements are no longer to be distinguished. The centrosome is likewise not to be distinguished from the chromatin mass near which it lies. A nuclear membrane is now formed about each daughter nucleus, which appears as a small vesicle with the chromatin mass at the polar side (Fig. 4, I). With the further development of the nuclear membrane the free cavity of the nucleus increases in size. The chromatin mass begins to swell, and is gradually transformed into threads and lumps which are arranged, at first, mostly along the nuclear membrane, but soon become distributed through the nuclear cavity. A nucleolus now appears, and with the further growth of the nucleus the chromatin passes over into the netlike framework like that in Fig. 4, J, A.

As soon as the nuclear membrane is formed, the polar radiations begin to disappear. In *Erysiphe* they seem to be transformed into a granular mass (Fig. 4, J). Finally, when the daughter nucleus is

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mature, the centrosphere remains as a much flattened disc closely applied to the nuclear membrane.

From the foregoing it is clear that, although differing much in detail, the karyokinetic process in *Erysiphe* is, in general, similar to that in the brown algæ. At our present state of knowledge, it is difficult to explain all the minor differences or to form an estimate of their relative importance.

MITOSIS IN POLLEN MOTHER-CELLS.

The spore mother-cells of certain *Liliaceæ* and other monocotyledonous species, as well as a few dicotyledonous plants such as *Helleborus* and *Podophyllum*, have become classical objects for cytological study, and in these genera the mitotic process is now as well understood as in any other angiosperms. The following discussion of the first two nuclear divisions in the spore mother-cells of higher plants is based upon the author's own investigations made upon *Lilium martagon*, *L. candidum*, *Fritillaria persica*, *Tradescantia virginica*, *Helleborus fœtidus* and *Podophyllum peltatum*.

THE FIRST OR HETEROTYPIC MITOSIS.

RESTING NUCLEUS AND DEVELOPMENT OF CHROMATIN SPIREM.

Soon after the last nuclear division in the archesporium, or spore-bearing tissue, which gives rise to the pollen mother-cells, the latter begin that period of growth so characteristic of spore mother-cells previously to the first mitosis. The nucleus is relatively large with a sharply defined membrane, and contains a fine linin network, in which the chromatin granules are held, and one or more nucleoli. The nucleolus may lie in a colorless, spherical cavity, which seems sharply circumscribed. The chromatin appears in larger and smaller granules, which are, as a rule, regularly distributed in the linin thread. The cytoplasm presents a uniform netlike structure (Fig. 5, A). This is the typical structure of a pollen mother-cell.

With further growth of the nucleus, the chromatin granules increase in size, probably through the union or aggregation of the smaller granules, while at the same time the linin thread contracts and shortens. In this stage the linin net consists of a complicated spirem or thread with short turns. The chromatin granules have attained a more uniform size, and lie more regularly distributed in the linin thread (Fig. 5, B). This contraction of the linin thread and fusion of the smaller chromatin granules continues, so that the nuclear thread, which later

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contains a row of larger granules or disks (the *Chromatinscheiben* of the German literature) of a tolerably uniform size, becomes a hollow spirem whose irregular turns traverse the nuclear cavity (Fig. 5, C).

The chromatin disks have usually a jagged or erosed outline, which shows that each disk is composed of smaller granules. The chromatin disks, first carefully described by Strasburger ('82), vary much among themselves in size, and do not always have the same orientation in the linin thread. This fact, together with the twisting of the thread upon its axis, which is a mechanical necessity, gives the impression of a spirem composed of very irregular granules. This is especially notice-

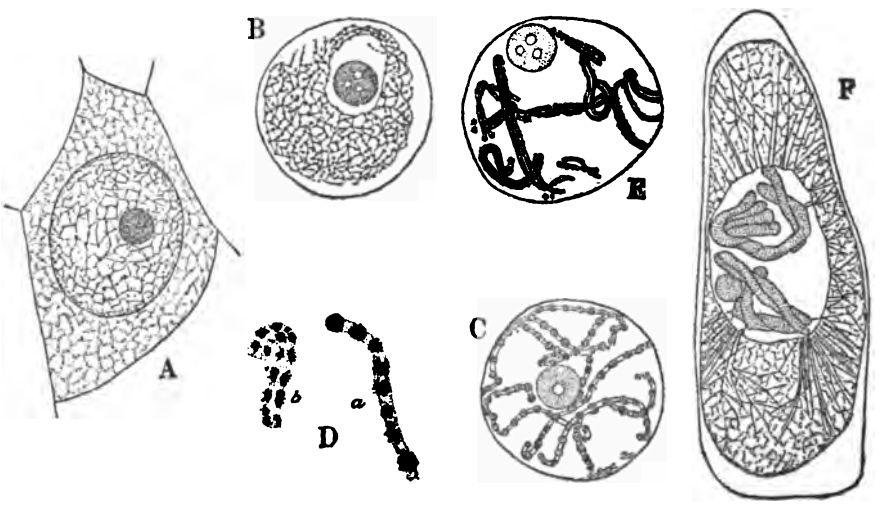

FIG. 5.—Pollen mother-cell and early prophase of first or heterotypic mitosis. A, F, *Podophyllum peltatum*. B–E, *Helleborus fœtidus*.

A, typical pollen mother-cell, with nucleus in resting stage, and while the cells are in tissue connection.
B, linin net with numerous small chromatin granules.
C, spirem in which chromatin disks are of uniform size.
D, pieces of chromatin spirem more highly magnified; *a*, before longitudinal splitting; *b*, after longitudinal splitting.
E, the spirem has split longitudinally; daughter segments show a tendency to separate.
F, the chromatin spirem has segmented transversely into chromosomes; daughter segments twisted about each other. (All figures represent sections.)

able immediately after the longitudinal splitting of the chromatin granules. At this stage the most careful staining is necessary to bring out the chromatin disks clearly, since the linin retains the stain with greater avidity, thereby concealing the former. If the nuclear thread be too densely stained, it will appear more or less homogeneous, in which case the chromatin disks manifest themselves as a succession of enlargements whose granular character is concealed. The chromatin thread consists, therefore, not of a succession of chromatin disks

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but of a continuous linin thread in which are held the chromatin disks or granules.

In an early stage the nuclear thread shows a marked tendency to contract into a ball or mass about the nucleolus. The contraction into a dense ball is regarded by some observers as a perfectly normal occurrence, to which the name *synapsis* has been given. My own investigations have convinced me that the contraction of the nuclear thread into a ball is in a large measure due to the reagents, and that synapsis has little or no significance. It indicates probably a very sensitive condition of the nuclear thread or net at the stage in which the contraction occurs.

Soon after the nuclear net has developed into the spirem, as mentioned, the chromatin and linin elements split longitudinally (Fig. 5, D, *a*, *b*, E). The daughter spirems remain either closely applied to each other, or, as sometimes happens, they may separate for longer or shorter intervals. They are always twisted upon each other, and, as a consequence, the two parallel rows of disks are not easily seen, especially where the chromatin thread makes short turns. The twisting of the daughter spirems upon each other persists after the transverse segmentation of the spirem into chromosomes, and in very many cases it is still to be seen during metakinesis (Figs. 6, 7).

Very frequently portions of the spirem which run parallel with each other are connected by very fine threads, and, in some cases, as in the pollen mother-cells of *Podophyllum*, very delicate cytoplasmic threads seem to penetrate the nuclear membrane and fasten themselves to the chromatin spirem. At this stage also one or more nucleoli, of varying sizes and with a homogeneous or vacuolate structure, are present. The nuclear membrane, especially in *Podophyllum*, does not present from now on the sharp contour of the resting nucleus. It seems to consist merely of a cytoplasmic boundary (Fig. 5, F), and as will be pointed out in a later paragraph, we may conclude that the nuclear membrane consists of an extremely delicate kinoplasmic network, whose meshes in the resting nucleus are so closely arranged that only a sharp line is seen when observed in optical section. As soon, however, as the meshes widen with the increase in size of the nucleus the nuclear membrane loses its sharp contour. It cannot be asserted with absolute certainty that the fine threads extending from the nuclear membrane to the chromatin thread penetrate the membrane and continue into the cytoplasm, but in *Podophyllum* the evidence seems to be in favor of such a view. At any rate there seems to be an intimate connection maintained between chromatin and cytoplasm.

As karyokinesis progresses, the chromatin thread contracts, becom-

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ing shorter and thicker, and frequently no trace of the longitudinal splitting can be recognized. There is thus formed the loose, hollow

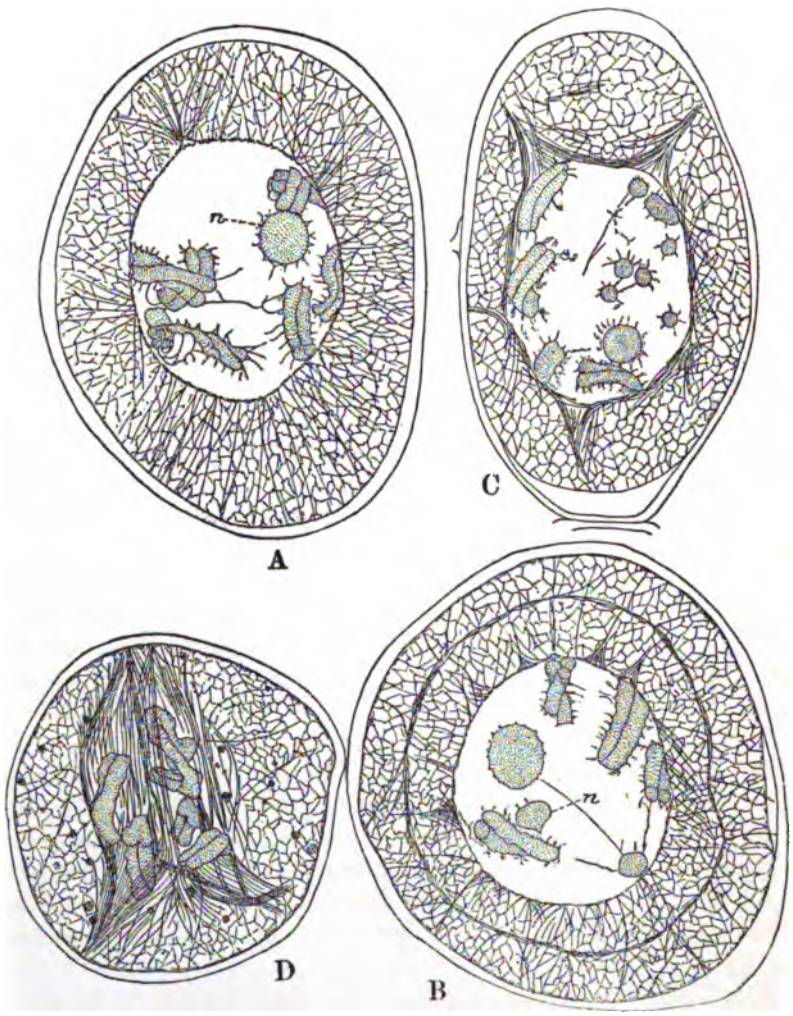

FIG. 6.—Prophase and early stages in development of spindle in heterotypic mitosis of pollen mother-cell. A, B, *Lilium candidum*. C, D, *L. martagon*.

A, the kinoplasmic spindle fibers arranged radially about the nucleus, large nucleolus present, and the chromosomes, each consisting of two rather thick segments twisted about each other, lie along the nuclear membrane or scattered through nuclear cavity.

B, same developmental stage as A; here the kinoplasmic fibers are disposed partly radially and partly in form of a weft lying in cytoplasm midway between nucleus and cell-wall.

C, the spindle fibers are encroaching upon the nucleus, forming a weft about it; the nuclear membrane as such has nearly disappeared; it seems to have been converted into fibers.

D, multipolar spindle complex, in which the chromosomes are irregularly distributed.

spirem, which segments by transverse division into the chromosomes.

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We shall now leave the chromosomes for the present and pass to the development of the spindle.

DEVELOPMENT OF THE SPINDLE.

The development of the spindle in pollen mother-cells varies somewhat in detail in different plants, but it can usually be referred to one type. In all cases, so far as known, it arises as a multipolar structure.

As soon as the spirem is segmented into chromosomes, and sometimes earlier, the kinoplasmic fibers make their appearance in the cytoplasm. The arrangement of the kinoplasmic fibers is not quite the same in all cells of the same anther. They may be disposed at first radially about the nucleus (Fig. 6, A), or, as in many cases, may form a weft about the nucleus midway between nuclear membrane and cell-wall (Fig. 6, B). The remaining cytoplasm consists of a fibrillar structure. In this stage the nucleus is filled with a fluid which does not stain, namely, the nuclear sap. The chromosomes are connected with each other and with the nuclear membrane by means of fine fibers, and one or more nucleoli are present. The nucleolus, however, begins to break up at this time, so that one large and several smaller ones may be present.

The next step in the development of the spindle may differ slightly in different cells, owing to the orientation of the kinoplasmic fibers. In those cells in which these fibers are disposed radially about the nucleus, the tendency to form poles manifests itself before the disappearance of the nuclear membrane. Groups of radiations converge toward various points near the plasma membrane, while others form a weft about the nucleus (Fig. 6, C). A little later the nuclear membrane is replaced by this weft, and the fibers begin to enter the nuclear cavity. In some cases well-defined poles (or only a few) are not as yet present. In other cases a greater number of poles are formed, and we have then a very remarkable multipolar complex of kinoplasmic fibers surrounding the nucleus, into which the fibers penetrate from all sides (Fig. 7, E).

Gradually more kinoplasmic fibers enter the nuclear cavity until it can no longer be recognized as such (Fig. 6, D). In this complex of spindle fibers the chromosomes are irregularly distributed. They are, however, soon collected together, and to each a bundle of fibers becomes attached. The chromosomes seem to be aggregated more closely together by a pushing and pulling of the spindle fibers. Owing to the irregular arrangement of the chromosomes and the complexity of the mass of spindle fibers, it is not always possible to determine at this stage the exact manner in which the fibers are fastened to the chromosomes (Fig. 7, F).

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The bipolarity of the multipolar spindle now gradually manifests itself, and the multipolar structure rapidly becomes a typical bipolar spindle in which the chromosomes are arranged in the equatorial plate.

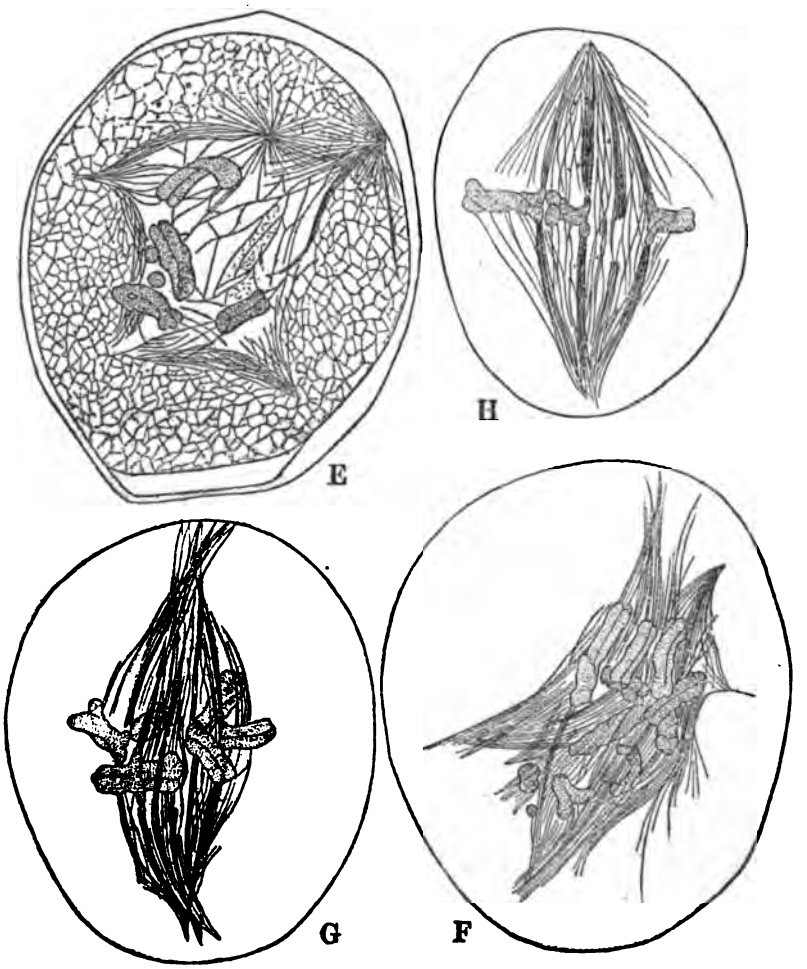

FIG. 7.—Heterotypic mitosis in pollen mother-cell (*L. martagon*). Development of spindle continued.
E, the weft of spindle fibers forms a multipolar complex.
F, a multipolar complex in which bipolarity has begun to manifest itself; the weaker poles seem to be drawn in or together.
G, bipolarity is established and chromosomes more regularly arranged in equator.
H, mature spindle, showing only 3 of the 12 chromosomes; chromosomes fastened endwise to spindle.

This transformation is probably brought about by certain of the larger poles converging toward a common area or point, while others are drawn in (Fig. 7, G). The mature spindle is either truncated at the poles (sometimes broadly so) or pointed, and the chromosomes are

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quite regularly arranged in the equatorial plate. They are usually radially disposed, standing at right angles to the axis of the spindle (Fig. 7, H). The spindle fibers present the following arrangement: to each chromosome are attached two bundles of fibers (one to each daughter segment) which extend to the poles; other fibers, the central spindle fibers, run uninterruptedly from pole to pole, and still others diverge from the poles toward the cell periphery. This arrangement is commonly found in all cells of the higher plants, whether they be reproductive or vegetative. The spindle does not, as may appear at the first glance, present a system of meridional fibers converging toward the poles, but, as is easily seen from thin sections, the fibers cross and anastomose, giving the impression that the spindle consists of a weft or complex of fibers drawn out in the direction of the poles, which, indeed, it really is.

In spore mother-cells of plants, the spindle fibers seem to be generally of cytoplasmic origin, *i. e.*, they appear first in the cytoplasm, forming a weft about the nucleus or radiating from it. In the generative cell of gymnosperms and in the first division following fecundation in these plants, it seems that the fibers or many of them arise from kinoplasm, which is in the nucleus or which entered the same in another form.

CHROMOSOMES.

As is well known, the chromatin spirem, which has split longitudinally in the early prophase, segments by transverse division into twelve chromosomes, the reduced number, or half the number in the vegetative cells of the sporophyte. Each chromosome consists, therefore, of two daughter segments, or daughter chromosomes, which are almost always twisted upon each other (Fig. 7, H; Fig. 8). After the segmentation of the spirem into chromosomes, these contract, thereby becoming shorter and thicker. Previous to the disappearance of the nuclear membrane, they lie near it or are scattered throughout the nuclear cavity (Fig. 6, B). In *Lilium*, the daughter chromosomes are, as a rule, closely applied to each other, but in many cases they tend to become separated soon after segmentation, so that various forms of chromosomes result, such as rings, loops, X- and V-shaped forms, depending upon the manner in which the daughter segments are oriented toward each other (Fig. 8, A to K). These various forms persist and may be found in the nuclear plate of the mature spindle.

The following will explain the manner in which the more frequently occurring forms are brought about in *Lilium*, *Podophyllum* and in many other higher plants:

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The daughter segments often diverge at one or at both ends (Fig. 8, B, C). In other cases they may be bent and in contact only near the middle (Fig. 8, D). If the daughter segments adhere at the ends, and bend away from each other near the middle, a ring results (Fig. 8, E). Ring-shaped chromosomes may be so bent as to bring the opposite ends near each other, in which case we have a ring partly folded upon itself. This is true in a measure in Fig. 8, E. When the segments forming a ring separate slightly at one end, an open ring is produced.

A Y-shaped chromosome will result when the segments are contiguous for a part of their length but diverge at one end (Fig. 8, F). Sometimes the daughter segments adhere near the middle but diverge

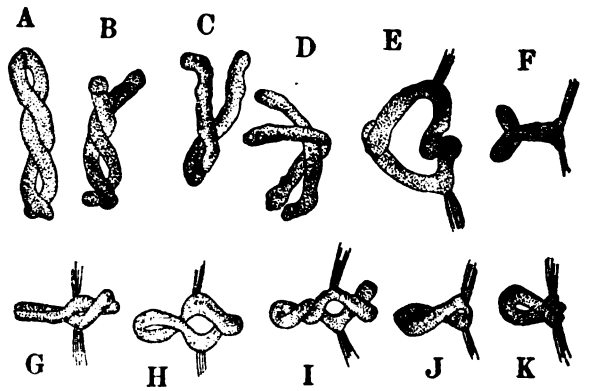

FIG. 8.—Heterotypic mitosis (*Lilium martagon*). Different forms of chromosomes.
A, B, C, D, chromosomes from prophase. E–K, from equatorial plate.
E, ring-shaped, F, Y-shaped, and J, typical X-shaped chromosomes.
G, H, I, and K, other forms commonly met with in *Lilium*.

at both ends, so that they may be crossed; this gives rise to the X-shaped chromosome (Fig. 8, J). Instances are also met with in which the segments of the X-shaped chromosome fuse completely at one end, and the chromosome appears as a continuous rod, folded in such a manner that the opposite ends are brought together. In this way loops and incomplete rings are produced (Fig. 8, K). In Fig. 8, G, H, and I are forms of chromosomes that are of frequent occurrence. The orientation of the daughter segments toward each other, which results in the different forms of chromosomes described, is, in all probability, of no special importance, since two or more of these forms may be seen in the same nucleus.

In *Tradescantia*, between the time of the segmentation of the spirem into chromosomes and the mature spindle, the daughter segments often contract into the form of short, thick crescents. These may adhere at

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the points of the crescents to form ring-like chromosomes (Fig. 9, D, at the right). In the majority of cases, however, they adhere at only one end, and under such circumstances each chromosome consists of two thick and slightly curved pieces placed end to end, and as they are oriented tangentially upon the spindle, reach nearly from pole to pole (Fig. 9, D).

The chromosomes in *Podophyllum* present the same variety of forms found in *Lilium* and *Tradescantia*. Here the segments may be in close contact, side by side, or form loops, rings, X's, and Y's. Perhaps the majority of chromosomes in *Podophyllum* present the form last mentioned for *Tradescantia*.

In *Lilium* the chromosomes, when in the nuclear plate, are usually arranged with much regularity about the periphery of the spindle. The majority are fastened to the fibers at the ends, and stand radially to the axis of the spindle (Fig. 7, H). When observed from the pole in this stage, they are seen to radiate like the spokes of a wheel from the central spindle fibers. But all the chromosomes are not so regularly oriented upon the spindle, and their manner of attachment to the fibers is also variable. As will be seen in Fig. 8, F–K, they may be fastened to the spindle at some distance from one end or near the middle. Those that are quite regularly ring-shaped are attached near the middle of each segment. In all these cases, the chromosomes are placed tangentially upon the spindle. The X-, Y-, and loop-shaped chromosomes are usually fastened to the spindle as indicated in Fig. 8, F, J, K. Karyokinetic figures are not rare in which two or more of the different forms of chromosomes, with their different orientations and different methods of attachment to the fibers, are found in the same spindle.[1]

The stage of the mature spindle persists some time and evidently

[1] Other interpretations of the chromosomes appearing in the first mitosis have been given by different observers and by the same investigator at different times, owing to the trend of theoretical considerations. One of these, which was announced as early as 1884 by Heuser for *Tradescantia virginica* (Beobachtung über Zellkerntheilung. Bot. Centralblt., 17: 1884) and which has very recently received support by Strasburger and others (Ueber Reduktionstheilung. Sitzbr. der Königl. Preuss. Akad. der Wiss., 18: 1–28, 1904) is that the two segments of each chromosome appearing in the equatorial plate of the first mitosis are not the result of the longitudinal splitting of the spirem occurring in the early prophase, but are formed by the folding together or approximation of two chromosomes, each consisting of the two daughter segments resulting from the longitudinal splitting. Each chromosome is therefore a bivalent chromosome, and the first or heterotypic mitosis is a qualitative or reducing division, whereas the second mitosis is equational, the segments separating along the line of the longitudinal split. Strasburger bases his conclusion mainly upon data obtained from studies of the pollen mother-cells of *Galtonia candicans*. The figures which he gives in support of this view in the paper cited seem to me to be far from convincing. Moreover, Jules Berghs, in a recent study of the prophase of the heterotypic mitosis in *Allium fistulosum* and *Lilium lancifolium* (*speciosum*) (La Cellule, 21: 173–188, 1904), shows clearly, in a careful series of stages, that the two segments of each chromosome are the result of the longitudinal fission and not that of a folding together or approximation of two chromosomes. Unfortunately the papers cited reach me too late for further consideration, as these pages are already in press.

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represents a slight pause in the process of mitosis. For this reason it is the stage most easily obtained and most frequently observed.

METAKINESIS.

Up to the stage of the mature spindle, as in Fig. 7, H, each chromosome is seen to consist of two daughter segments oriented in one of the ways described above. As soon, however, as these segments begin to separate in metakinesis, each splits longitudinally in a plane at right angles to the longitudinal splitting which took place in the prophase. In some instances, and when the chromosomes are viewed from the end, each is seen to be composed of four rods, the four granddaughter segments, placed side by side in pairs, forming a tetrad, Fig. 9, A. As a rule the granddaughter segments cannot be definitely recognized until the daughter segments have separated somewhat. Having almost or quite separated, the daughter segments are seen to be in the form of a V, although it never should be forgotten that V's do not invariably result. As the result of the second longitudinal splitting, each typical V-shaped daughter chromosome consists of two granddaughter segments which adhere or are even fused at the ends to which the spindle fibers are fastened, while the opposite ends diverge (Fig. 9, B). It frequently happens that the opposite ends of the granddaughter segments do not diverge, but lie more or less in contact side by side, so that the retreating daughter chromosomes consist of two applied rods (Fig. 9, F, the middle pairs). In some cases, as already mentioned, the ends of the granddaughter segments forming the angle of the V fuse, so that the V appears to be one piece formed by bending. The bent or contorted condition of the granddaughter segments during metakinesis is due to the previous twisting of the daughter chromosomes upon each other.

If the chromosomes be in the form of rings, as shown in Fig. 8, E, it is evident that the separating daughter chromosomes may also be in the form of a V or U, but such V's and U's will be produced by a bending of the daughter segments. This is true in a great many cases in *Lilium* and in other plants, among both monocotyledonous and dicotyledonous species. In such cases each U or V is invariably double, as the result of the second longitudinal fission—that is, the granddaughter segments are U-shaped and closely applied to each other (Fig. 9, F, right and left). Sometimes these granddaughter segments may separate slightly, giving the impression of two similar daughter chromosomes lying one just beneath the other. This is one of the several phenomena that have led to erroneous interpretations of the chromosomes.

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In Fig. 9, C, on the left, is shown a chromosome in metakinesis, which is fastened to the spindle near the middle. Each daughter segment, which is split longitudinally, is in the form of a U-like figure,

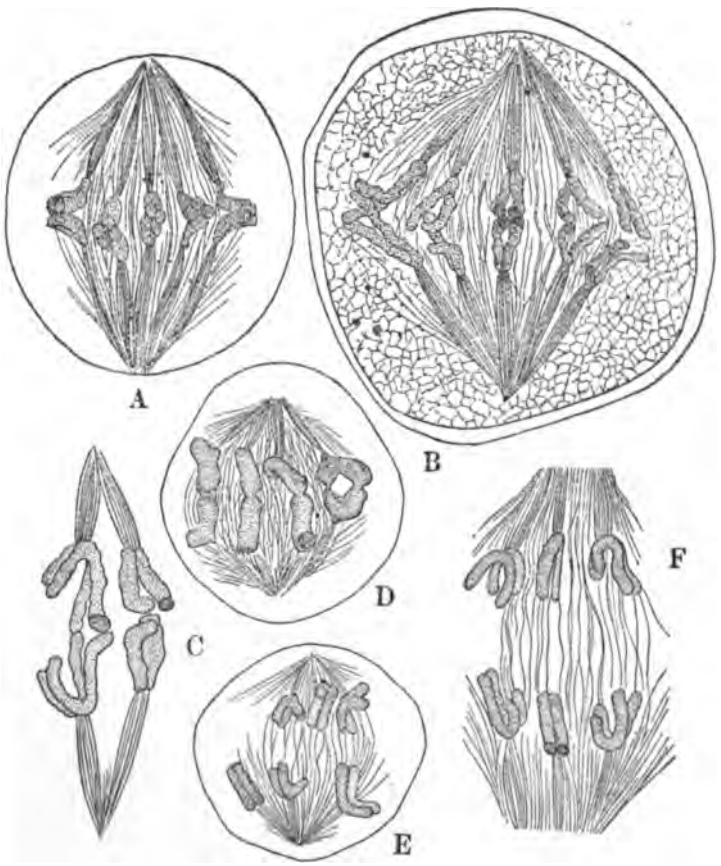

FIG. 9.—Heterotypic mitosis. Meta- and anaphases. A, B, C, and F, *Lilium*. D, *Tradescantia*. E, *Podophyllum*.

A, metakinesis beginning; viewed from the end, each chromosome is seen to consist of four rods, due to the second longitudinal splitting, which has taken place at right angles to the first.

B, metakinesis accomplished; ends of granddaughter chromosomes, which are directed toward equator, diverge, giving rise to the well-known V-shaped elements; in B all chromosomes are fastened to spindle fibers at the ends.

C, chromosome on left was in form of an incomplete ring; segments fastened at place of bending; in this case the U- or V-shaped elements owe their form to a bending; the chromosome on the right was attached endwise.

D, mature spindle of *Tradescantia*. E, F, anaphase; the retreating pairs of granddaughter segments are rods hooked at one end, or U's.

in which one limb seems a little longer than the other. This chromosome may originally have been a complete ring, as in Fig. 8, E, in which the segments had separated at one end in advance of the other,

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or it may have had this form at an earlier stage. The chromosome at the right in this figure (Fig. 9, C), was attached to the spindle endwise, and the retreating granddaughter segments will probably form V's. If the chromosome on the left were rotated 45°, so that the segments would be seen in profile, we might have the picture of two double V's or U's about to separate, for, as shown in the figure, the free ends of the pairs of granddaughter elements tend sometimes to diverge. The two chromosomes in this figure, which belong to the same spindle, show clearly how figures of the same shape may be produced in different ways. In the one on the right the chromosome was probably attached to the spindle by the end, and the V's are formed by the divergence of the free ends, while that on the left was fastened near the middle of each segment, and the V- or U-shape of the retreating segments is the result of a bending.

In such chromosomes as Fig. 8, G, H, I, the retreating elements may retain their present form, or they may be bent during metakinesis into U's or V's. When the daughter segments of such chromosomes are separated, they must untwist, and it is reasonable to suppose that the force necessary to separate them when twisted will be sufficient to bend the segments into a U- or V-like figure.

THE ANAPHASE.

The pairs of granddaughter segments, as they pass toward the poles, are in the form of contiguous, straight, or undulating rods, V's or U's, or, in case one limb of the last two named figures be much longer than the other, as is sometimes observed, the retreating elements will be in the form of hooks. Even in those cases in which both granddaughter segments are nearly straight or undulating rods of equal length, each is often slightly bent or hooked at the end fastened to the spindle fibers, or the segments may be bent at both ends.

The daughter chromosomes in *Podophyllum* and *Tradescantia* show with great clearness their double character during the anaphase (Fig. 9, E). The granddaughter segments generally lie close side by side, although cases in which they are slightly separated are now and then to be observed. There are in these genera also variations in the forms of the chromosomes which may be explained in the same manner as in *Lilium*.

The retreating chromosomes and the structure of the spindle suggest that the segments are conveyed to the poles by a pushing and pulling action of the spindle fibers.

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THE TELOPHASE.

As soon as the daughter chromosomes arrive at the poles, they approach each other very closely, so that, in many cases, the separate individuals cannot be recognized. But very frequently the segments do not become so closely crowded together, and the manner in which the daughter spirem is formed can be followed with accuracy. The formation of the spirem can best be observed when the granddaughter segments arrive at the poles in the form of the familiar V-shaped figures. Generally the ends forming the angles of the V fuse first, unless this has already been accomplished; then the free ends meet end to end and unite (Fig. 10, G). In this way there is formed a continuous single spirem in which the identity of the individual segments or granddaughter chromosomes is lost.

If all the daughter chromosomes were regularly V- or U-shaped the spirem would be regular, consisting of an orderly series of nearly uniform turns; but the spirem rarely shows such regularity, because the chromosomes vary in size and shape and in the manner in which the granddaughter segments are oriented with respect to each other in the several pairs. During the reconstruction of the daughter nucleus, the chromosomes tend to reticulate, that is, to become irregular and lumpy, so that an irregular skein or net results. This is less pronounced in *Lilium* than in many other plants.

The fact that pairs of granddaughter segments arrive at the poles in different forms, such as V's, double U's, and pairs of parallel rods, shows clearly that in such cases the resulting spirem must be very irregular. The chromosomes are generally so closely crowded together that it is not possible to determine with certainty just how the variously shaped pairs of segments behave. But it is reasonable to suppose that the segments of the double U's and those of contiguous rods must first separate in order to unite end to end, for no case has been clearly made out in *Lilium* in which a part of the spirem is formed double.

The newly formed daughter spirem is close with relatively short turns (Fig. 10, G, H). Between each two extends the beautiful system of connecting fibers, which represents the central fibers of the spindle. Fibers are also present which extend from each spirem toward the plasma membrane in the direction of the equator. Some of these reach the plasma membrane, while others seem to end blindly in the cytoplasm, or pass over into its thread-work. In *Lilium* there are no polar radiations.

The system of connecting fibers soon becomes barrel-shaped, and the cell-plate makes its appearance in the equatorial region. We shall return to the formation of the cell-plate beyond.

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The nuclear membranes are not formed about the daughter nuclei in *Lilium martagon* until after the division of the cell, at least in many instances. Soon after the division of the cell, however, the nuclear membranes are laid down. In all plants examined, each appears first

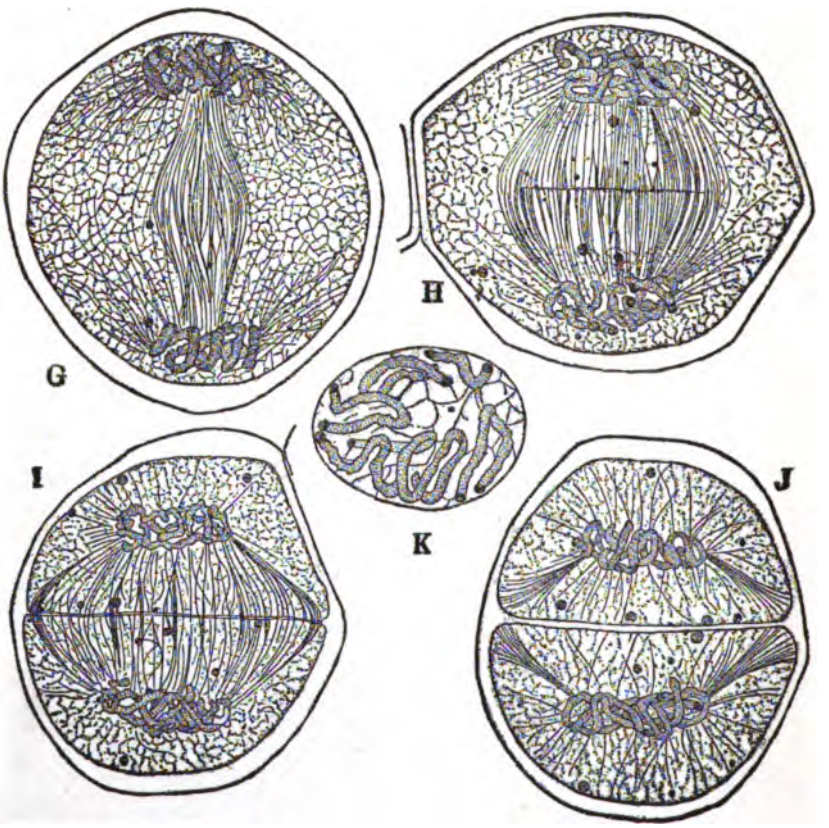

FIG. 10.—Telophase and daughter nucleus of heterotypic mitosis (*Lilium martagon*).

G, daughter spirem formed by union of granddaughter segments end to end; each daughter spirem is in the form of a disk from whose edges kinoplasmic fibers extend out in direction of cell-wall; system of connecting fibers slightly bulged out at middle.

H, the cell-plate appears in center of system of connecting fibers.

I, J, cell-division is completed, but the daughter nuclei are not yet provided with membranes.

K, a daughter nucleus at a later stage with nuclear membrane; chromatin spirem continuous, the free ends having been made by knife in sectioning.

as a weft of kinoplasmic fibers, which are undoubtedly derived from the spindle. It is interesting to note that in *Lilium* and *Podophyllum* the nuclear membrane appears in the same form in which it disappeared during the formation of the spindle. The fact that the nuclear membrane arises first as a weft of kinoplasmic fibers is a strong proof that it is of a kinoplasmic nature.

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The young weft-like nuclear membrane encloses a cavity containing the chromatin and little or no other staining material. With further development the kinoplasmic weft is transformed into the typical nuclear membrane, appearing in section as a sharp line, and the daughter spirem becomes loose and open. In the mature daughter nucleus the spirem is continuous and of a tolerably uniform thickness. In some cases it is rather regular, consisting of long turns arranged in the form of a wreath (Fig. 10, K), but in the majority of instances the spirem is irregular, with long and short turns so disposed that its course cannot be easily followed. This condition of the spirem is in all probability due to the variously shaped chromosomes mentioned in a preceding paragraph.

THE NUCLEOLUS.

In the resting nucleus and during the prophase, one or more nucleoli are present. These nucleoli take on a deep red or reddish purple color with the Flemming triple stain. They sometimes present a uniform structure, but, as a rule, the larger nucleoli especially reveal one or more vacuoles. As has been mentioned in a preceding paragraph, the nucleolus very frequently lies within a spherical space which appears in optical section as a colorless court about it. This phenomenon is especially striking in vegetative cells of higher plants, such as in root tips of *Vicia faba* and *Zea mays*. Experiments seem to show that the colorless space surrounding the nucleolus contains something more than a mere watery fluid which is extracted in dehydration. By subjecting roots of *Vicia*, *Zea* and others to a strong centrifugal force, the author (Mottier, '99) found that the nucleolus together with its surrounding colorless court was thrown out of the nucleus into the cytoplasm. The expelled nucleolus was still surrounded by its colorless court—a fact that seems to show that the colorless substance has a specific gravity much greater than other constituents of the nucleolus, and that it may be provided with its own membrane. This colorless substance may represent unorganized nucleolar matter.

Frequently before the nuclear membrane disappears a disorganization begins by which the nucleolus is broken up into several smaller nucleoli (Fig. 6, C). As the nuclear membrane fades away, and the kinoplasmic fibers enter the nuclear cavity, numerous bodies are found distributed in the cytoplasm which stain exactly as nucleoli, and there is no doubt that these bodies represent nucleolar substance. These extra-nuclear nucleoli were found to be more abundant in *Lilium martagon*. In *Lilium candidum* there may be none, or only a few

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small ones, at corresponding stages of mitosis. The presence or absence of extra-nuclear nucleoli may not depend so much upon the plant, perhaps, as upon the condition or activity of the cell. From the spindle stage of the first to the end of the second division there is no noticeable regularity in the behavior of these bodies. In different cells in the same stage of mitosis they may be present or wholly wanting. Even after the daughter nuclei are provided with membranes, and a nucleolus is present in each, extra-nuclear nucleoli are to be frequently seen in the cytoplasm. The same holds also for the second mitosis. A careful investigation of the behavior of the nucleolus in both *Thallophyta* and higher plants has shown that the nucleolus appearing in the daughter nucleus is not one of the extra-nuclear nucleoli which happened to lie near the chromatin, or in such a position as to be included by the nuclear membrane, but that the nucleolus arises anew in each daughter nucleus. The nucleolus appearing in the daughter nucleus arises usually near or in contact with the chromatin thread, but it is not implied that the nucleolus represents reserve chromatin.

In the higher plants and in those with typical nuclei the morphological evidence furnished by a study of karyokinesis, as well as the evidence of experimental physiology, goes to show that the nucleolus in such plant cells represents so much food material which can be drawn upon by the cell according to its needs. Whenever the activity of the cell is more intense, the nucleolar substance tends to become diminished, and it matters not whether the activity is directed toward constructive work or the production of energy. It is true that in some cases the food material furnished by the nucleolus seems to be used in a large measure by the chromatin, for example, in *Dictyota*, but in others by other parts of the living substance, as in the growth of the spindle or cell plate. In certain species of *Spirogyra* (Wisselingh, '98), in which, as it has been claimed by several investigators, the nucleolus furnishes directly one or more chromosomes, greater difficulties present themselves. It is not improbable that the nucleolus of such plants as *Spirogyra* may possess a totally different composition from that of the typical nucleolus, and we may, therefore, speak with propriety of *chromatin nucleoli.* However the behavior of the nucleolus is not well enough known in the plant kingdom to justify any attempt to harmonize all the facts now known. Applied to the higher plants the above conclusion seems to be very reasonable, since the facts there are almost wholly confirmatory.

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THE SECOND OR HOMOTYPIC MITOSIS.

In the pollen mother-cell of *Lilium*, the daughter nucleus does not pass into the complete resting stage, although in some cases the chromatin tends to become reticulated. In the homologous division in the embryo-sac, the daughter nucleus, on the contrary, passes into a structure which approaches closely that of the resting condition. In *Tradescantia* the chromatin of the daughter nucleus reticulates more than in *Lilium* while in certain dicotyledonous species, *e. g.*, *Liriodendron* and *Magnolia* (Andrews, '01), a complete resting condition is reached.

The spindle in *Lilium* and in all other plants investigated by the author arises also as a multipolar complex of fibers. The development of the multipolar structure and its transformation into the typical bipolar spindle differ in no essential from that already described for the first mitosis.

In *Lilium*, it is very evident that the spirem does not segment completely into chromosomes before the disappearance of the nuclear membrane. The spirem does not split longitudinally in this division, since that part of the process was accomplished in the preceding mitosis, but during the transformation of the multipolar into the bipolar spindle the chromatin skein segments into the chromosomes, which are arranged in pairs in the nuclear plate.

Within the complex of spindle fibers, the spirem, or pieces of it, provided it has partly segmented, are somewhat crowded together. The various turns are greatly entangled, kinked and knotted, so that the segments cannot be accurately traced out. In only the most favorable cases at this stage can a few segments or parts of the spirem be followed definitely throughout their entire length (Fig. 11, A). The kinked and entangled condition of the skein or its segments is due doubtless to the irregularity of the spirem, for were the turns all of a uniform shape and size a less complicated arrangement would result. The appearance of the chromatin during the development of the spindle suggests that the chromosomes were brought to a more regular arrangement in the nuclear plate by a pushing and pulling of the fibers.

Judging from the form of certain chromosomes which stand out by themselves, and which can be traced throughout their entire length during the development of the spindle or in the nuclear plate, it seems that the spirem, or a part of it at least, segments into pieces comprising the two segments of a chromosome, *i. e.*, the two granddaughter chromosomes of the first division, and that these pieces may correspond to long turns or loops of the spirem (Fig. 11, B, C). These loops are

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fastened to the fibers either at the free ends or at the place of bending. Now, in order that the two segments of such a chromosome may come into contact side by side, as is frequently the case, the parallel parts of the loop need only be brought closely together. This may happen

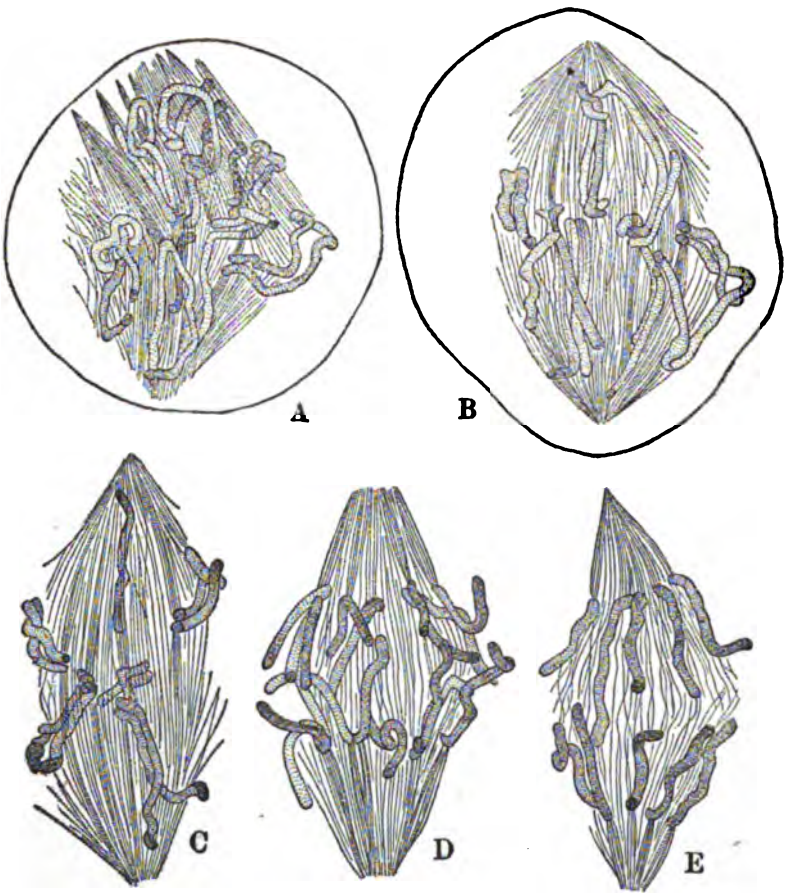

FIG. 11.—Second, or homotypic mitosis, in pollen mother-cells (*Lilium*).

A, multipolar stage of spindle; chromatin spirem not completely segmented into chromosomes.
B, bipolarity established; chromosomes more regularly arranged.
C, mature spindle; chromosomes more regularly disposed in equator, placed radially or tangentially on spindle.
D, metakinesis. E, the anaphase.

before the spirem is completely segmented, for in many chromosomes the two segments are very closely applied and twisted upon each other before the spindle is mature. In like manner parallel portions of the spirem may come in contact either before or after segmentation. In many other cases, both in the mature spindle and during its develop-

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ment, the two segments are separated from each other, being in contact only at the ends which are attached to the spindle fibers. Under this circumstance one segment may lie tangentially on one side of the equator and the other on the other. Other instances are observed also in which the two segments may lie parallel in pairs, but not in contact when arranged in the nuclear plate or at an earlier stage. Such cases as the two last mentioned would seem to indicate that the spirem, or a part of it, is segmented into the granddaughter chromosomes, and that these are then brought together in pairs. It is also probable that pieces of the segmented spirem, which are nearly straight, or only a little curved, may consist of two granddaughter segments, and these are brought side by side by the folding of the piece at or near the middle, so that the free ends are brought into apposition, after which the piece is severed at the point of bending. From a careful study of the second mitosis in the pollen mother-cells of *Lilium*, *Podophyllum*, *Tradescantia* and others, the author is inclined to believe that the spirem may segment in the different ways just mentioned. However, the daughter spirem segments transversely into the granddaughter chromosomes, and during the development of the spindle these are arranged more or less in pairs in the nuclear plate (Fig. 11, C).

In the nuclear plate, the chromosomes are oriented either radially, obliquely, or tangentially to the major axis of the spindle. The segments may be straight or variously bent, and, in either case, frequently twisted upon each other. In *Lilium*, the segments are frequently, perhaps in the majority of cases, variously twisted, kinked or knotted, so that they can be followed for only a part of their length. In many cases, the kinked and twisted chromosomes seem to be so contracted as to form lumps. This is true also in *Tradescantia* and in numerous other plants. The bent, kinked, and twisted condition of the chromosomes seems to be due to the irregularity of the spirem, for it seems probable that, were all the turns of the chromatin skein regular and uniform, the greatly entangled nature of the spirem would not appear during the development of the spindle.

We have seen that the identity of the individual chromosomes is lost from observation in the daughter spirem, and the question bearing upon the theory of the individuality of the chromosomes, naturally arises as to whether the chromosomes of the second, or homotypic mitosis, are identical with the pairs of granddaughter segments of the anaphase of the preceding, or heterotypic division. In other words, are the two segments of each chromosome, appearing in the nuclear plate of the second nuclear division, sisters? Or may it be possible that some are sisters, while others are composed of segments from different pairs of granddaughter chromosomes of the first division?

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It is generally conceded that the segments of each chromosome are sisters, and it is conceivable that, no matter in what manner or when the daughter spirem may segment during division, the spindle fibers, or those parts of the cell which have to do with the arrangement of the chromosomes in the nuclear plate, are able to bring the sister segments together in pairs.

Strasburger, Guignard, and others regard each long loop or turn of the daughter spirem as representing a V or U of the preceding mitosis, and that, consequently, the spirem segments exactly as it was constructed, *i. e.*, the chromosomes simply separate at the points marking the free ends of the V's and U's. The spirem accordingly breaks up into pieces equal to the length of two segments or two granddaughter chromosomes. It is claimed by Strasburger (1900, pp. 23, 24) that these V's or U's are fastened to the spindle in the same manner as in the first division, namely, at the angles or at the place of bending.

Theoretically, there may be little objection to this view. The vast majority of facts, however, show that there is no such regularity in the shape of the chromosomes, or in their manner of attachment to the spindle. We have seen that, in the daughter nucleus, the identity of the individual chromosomes cannot be recognized, and we do not know whether the spirem segments in the same manner in which it was constructed.

But if the spirem should segment by transverse division at the points marking the angles of the V-shaped chromosomes instead of at the free ends, then it is clear that the two segments of each chromosome would not be sisters. The result might be that two or more sister chromosomes would go to the same daughter nucleus, a condition that might furnish a basis for greater variation. We cannot prove either proposition, and the author is not disposed to enter into any speculation here upon the subject. The observed facts are these: The identity of the individual chromosomes is lost in the daughter nucleus, and we do not know whether the segments of the respective chromosomes appearing in the nuclear plate of the second mitosis are sisters or not. There is also no basis in fact for the conclusion that one chromosome is hereditarily different from another.

The first two nuclear divisions in the embryo-sac mother-cell, so far as is known, are quite similar and homologous to those in the pollen mother-cell. In *Lilium martagon*, the species more carefully investigated by the author, there is no important difference in the behavior of the chromosomes. It may be mentioned, moreover, that the daughter nuclei resulting from the first mitosis approach more closely the resting condition than in the pollen mother-cell.

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The question now remains whether in all micro- and macro-spore mother-cells of the higher plants a double longitudinal splitting of the chromatin takes place during the first mitosis and how prevalent such a phenomenon is in both plants and animals.

In those plants in which the daughter nucleus passes into the structure of the complete resting stage, it is certainly difficult to understand the significance of the double longitudinal splitting of the chromosomes in the first division.

CELL-DIVISION.

THE TYPE OF THE HIGHER PLANTS.

Modern research has established the very important fact that new cells are formed from uninucleate or multinucleate mother-cells according to different methods, depending largely upon the manner in which the new plasma membranes differentiating the cells are formed.

(1.) Among the higher plants, and some *Thallophyta* as well, in which cell-division is generally intimately associated with nuclear division, the new plasma membrane or membranes are laid down through the instrumentality of kinoplasmic connecting fibers, extending between the nuclei concerned.

(2.) In the ascus of certain *Ascomycetes*, where the new cells (spores) are carved out of a common nucleated mass of cytoplasm or mother-cell, the plasma membrane is also formed by kinoplasmic fibers, but these are polar radiations and not connecting fibers. The entire plasma membrane of such cells is new, that of the mother-cell taking no part in the process. This is typical and real *free cell-formation*.

(3.) Another form of cell-division is found among the *Myxomycetes* and certain *Phycomycetes*, in which the new plasma membranes arise by a process of progressive cleavage, beginning at the surface, with or without any connection with, or aid of, vacuoles. Kinoplasmic connecting fibers or radiations are in no way connected with this process. This type we may know as *cell-cleavage*. It resembles the cleavage of animal cells more closely than do the other processes of cell-formation in plants.

(4.) There is yet another method of cell-formation typified by *Dictyota* and *Stypocaulon* among the brown algæ, in which the new plasma membrane seems to be a direct transformation of the meshes or threadwork of the cytoplasm. It is not a cleavage like the last mentioned, nor are any connecting fibers present to take part in the

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formation of the cell-plate. This method is, however, closely related to cleavage.

As an illustration of the method of cell-plate formation typical of higher plants, the pollen mother-cells of *Lilium* furnish excellent material. Here a cell-division follows the first nuclear division. The connecting fibers are well developed, and with suitable fixing and staining the details stand out with a clearness unequaled among plants. As we have seen in Fig. 10, G, the daughter spirems are connected by a beautiful system of connecting fibers, which is slightly barrel-shaped at an early stage. The fibers soon show a thickening in the equatorial region, which stains more intensely with gentian violet. The thickenings are not granular or lumpy, but rather homogeneous, and are due to the accumulation of kinoplasm, the substance out of which the cell-plate, or plasma membrane, is made. At a little later stage (Fig. 10, H) there appears in the central part of the system of connecting fibers in the region of the equator a fine homogeneous line, the beginning of the cell-plate. This young cell-plate is evidently in the form of a circular disk, which proceeds in growth uniformly toward the periphery of the cell. The cell-plate is not necessarily formed by the meeting or union of thickened places of the connecting fibers, for in many cases the fibers are too far apart. The kinoplasmic material is brought to the place occupied by the new plasma membrane and there deposited in the form of a fluid substance. With the further growth of the cell-plate the connecting fibers bulge out more and more, being always thicker and more numerous at the outer edge or surface of the system (Fig. 10, H). As the peripheral fibers of the barrel-shaped system bulge out, its longitudinal axis becomes shorter, so that the daughter spirems come eventually to lie in the center of the daughter cells. In Fig. 10, I, the cell-plate is just complete, the peripheral fibers which have reached the plasma membrane of the cell being more numerous there.

The cell-plate or plasma membrane is now seen to be double, and it is the author's opinion that the new plasma membrane is formed double. The fact that each daughter or granddaughter cell, when somewhat shrunken at this stage, is seen to possess its own plasma membrane, seems to support this view.

Soon after the formation of the plasma membranes, a cell-wall is deposited between them. Until the primordia of the daughter nuclei (Fig. 10, J) are provided with a nuclear membrane, the chromatin spirem is in the form of a circular disk from whose margin radiates a zone of kinoplasmic fibers toward the equatorial edge of the cell. In optical section this zone appears as a bundle of fibers on the right and

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left, whose elements diverge, meeting the concave plasma membrane at different points. Other delicate fibers extend from the spirem in all directions toward the plasma membrane. As soon as the nuclear membrane appears these radiating fibers become more uniformly distributed about the nucleus. They undoubtedly take part in the formation of the spindle in the division of the daughter nucleus.

FREE CELL-FORMATION.

The most beautiful and best known illustration of typical free cell-formation is found in the development of the spores in the ascus of certain *Ascomycetes* as described by Harper.

The delimination of the spores from the cytoplasm in *Erysiphe* follows immediately after the close of the last of the three successive nuclear divisions which furnish the eight nuclei for the spores. The entire process is accomplished by those kinoplasmic fibers which constitute the polar radiations of the last nuclear division and in a manner quite peculiar to asci.

All of the eight nuclei pass through the anaphase at the same time, and, when in the resting condition, cannot be distinguished one from the other, with the exception of those that lie close to the wall. The polar radiations persist in connection with those nuclei that form spores, while from those which do not the radiations disappear entirely. The chromatin lies mostly free in the nuclear cavity, but it is always in communication with the nuclear membrane, especially near the centrosphere (Fig. 12, A). As the first indication of cell-formation, the nucleus becomes pointed and develops a beak-like prolongation on the side next to the pole or centrosphere. This point or beak gradually elongates, so that the centrosphere becomes farther removed from the body of the nucleus (Fig. 12, B). As soon as the beak reaches a length which exceeds slightly the diameter of the nucleus, its growth ceases. This beak consists not of a single fiber or thread but of a slender cylindrical tube arising abruptly from a rather broad base. Into the tube there extends quite to the centrosphere a continuation of the chromatin net, by which the latter remains in communication with the centrosphere. In the base of the beak the nuclear network is loose and more open, while in the slender part it is drawn out into a single and twisted thread.

As soon as the beak has reached its definitive length the kinoplasmic radiations undergo a remarkable change. The radiations which have a direction similar to that of the beak begin now to bend or grow backward, with the centrosome as a center, toward the nucleus, so that

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the aster is converted into a hollow cone whose apex is the centrosphere. Neighboring radiations unite and grow rapidly in length, at the same time bending back toward the nucleus in a manner resembling the spray from a fountain. An optical section of this stage is shown in Fig. 12, C. With further growth the kinoplasmic rays give rise to a sort of bell-shaped or half-ellipsoidal structure whose center is occupied by the nucleus and whose pole is formed by the centrosome (Fig. 12, D). Near the centrosome the fibers have already formed a continuous but extremely thin layer, the plasma membrane, separating the cytoplasm of the spore from that of the ascus. At the edge of

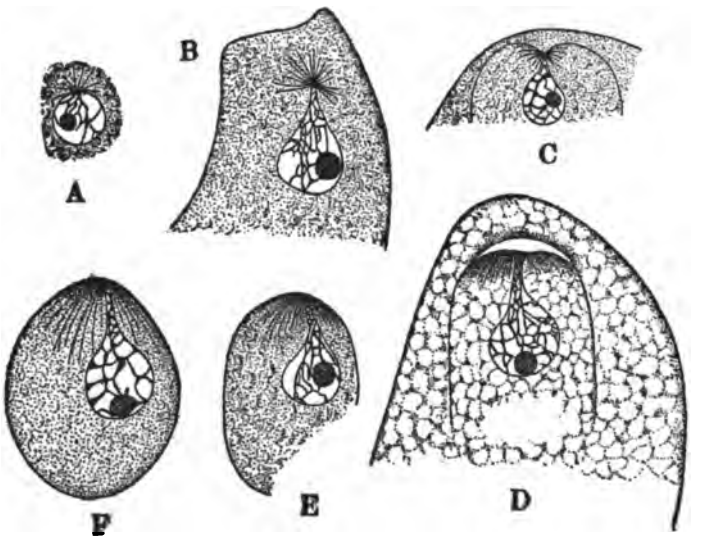

FIG. 12.—Free cell-formation in ascus of *Erysiphe communis*.

A, nucleus with centrosphere.
B, development of nuclear beak.
C, polar radiations extend outward and backward as spray from a fountain.
D, formation of plasma membrane from end of beak outward, and continued growth of kinoplasmic fibers backward.
E, F, meeting of fibers at opposite end of ellipsoidal spore and establishment of a complete plasma membrane delimiting spore-plasma from remaining plasma of ascus.—(After Harper.)

the bell the radiations end as free fibers, continuing their growth, however, in a direction corresponding to the periphery of the ellipsoid (Fig. 12, E). Finally these fibers meet in a point which is directly opposite the centrosome, and unite end to end and laterally. The formation of the plasma membrane continues, so that eventually an ellipsoidal or oval cell is delimited from the cytoplasm of the ascus by a complete plasma membrane (Fig. 12, F). At first the plasma membrane is thicker near the centrosome, but later its thickness becomes uniform throughout.

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Fig. 13, I, J, shows several stages of the process just described in two asci of *Lachnea scutellata*.

While this is taking place the nuclear beak becomes smaller and smaller until it is finally reduced to a mere thread in which chromatin and membrane are no longer recognizable. The centrosome remains for a short time as a deeply staining and sharply defined disk adhering to the plasma membrane. Very soon it becomes free from the membrane and is drawn back to the somewhat pointed nucleus, where it appears as a saddle-like thickening upon the point of the nucleus, or

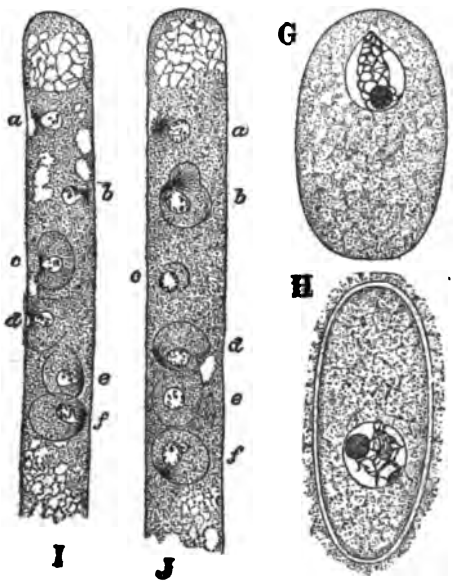

FIG. 13.—Free cell-formation in the ascus.

G, H, *Erysiphe communis*. I, J, *Lachnea scutellata*.

G, the plasma membrane is complete; nuclear beak withdrawn and centrosome saddle-shaped, and closely applied to the nuclear membrane.

H, a mature spore with cell-wall; centrosome closely applied to nuclear membrane at upper side.

I, J, portions of two asci showing several steps in process of free cell-formation *in situ*.—(After Harper.)

as a simple disk (Fig. 13, G, H). The nucleus now gradually assumes its original spherical form, the chromatin passing into the structure of the resting stage, while the centrosome remains closely adhering to the nuclear membrane.

It will be observed that in the specific case of cell-formation described the plasma membrane is completed before the nucleus has reached the resting stage, but in *Lachnea* (Harper, 1900) the daughter nuclei of the eight-nucleated stage are completely reconstructed before the beaks are formed. This may be, of course, a case of individual variation and of only secondary importance.

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CELL-CLEAVAGE.

The process of cell-formation by means of a progressive cleavage is best known at present in certain *Phycomycetes* and *Myxomycetes*. As a convenient and suitable illustration of this method the process of cleavage leading to spore formation in the sporangium of *Synchitrium*, parasitic upon the hog peanut, and of *Sporodinia* is selected. For our knowledge of cleavage we are again indebted to the researches of Harper ('99).

The so-called initial cell of the sporangium of *Synchitrium*, when almost fully developed, is large enough to be visible to the unaided eye, and contains a relatively large nucleus (Fig. 14, A). This nucleus divides several times until a large number of nuclei are present, which lie irregularly distributed in the cytoplasm.

Cleavage of the cytoplasm now begins. It does not take place by repeated bipartitions, nor by the simultaneous precipitation of a cell-wall about each nucleus. As mentioned in a preceding paragraph, it resembles in a large measure the process in certain animals, as for example, the dividing protoplasm of the germinal disk of the chick, or perhaps more nearly that in certain insect eggs in which a series of nuclear divisions precedes cytoplasmic segmentation.[1]

The cleavage begins by the formation of furrows on the surface, which grow deeper and deeper in a direction more or less radial. It is progressive and divides the cell into successively smaller portions (Fig. 14, D). The process is described in detail by Harper as follows:

> These grooves are in reality so narrow as to appear as plates, which grow wider by additions along their inner margins till they intersect, and thus divide the protoplasm into irregular blocks or sometimes pyramids with their bases in the surface of the initial cell (Fig. 14, D, E). Only at the very periphery the separation of the cut surfaces of the protoplasm to form a shallow notch, as it appears in section, reveals the true nature of the process as a pushing in of the free surface to form a deep though extremely narrow constriction.
>
> In many cases there is at first no separation of the newly formed surfaces; they remain closely appressed, up to the periphery of the cell. The groove appears in section, merely as a single line which the Zeiss appochromatic lens 1.40 ap. fails to resolve into two closely appressed surfaces (Fig. 14, B). The position of the line is further emphasized by the arrangement of the vacuoles, which are pushed aside and form in section two more or less regular rows in the plane of the newly formed surfaces on each side of the furrow. Such a line might be taken for a cell-plate which subsequently splits to form the boundaries of the protoplasmic segments or which is metamorphosed into the cellulose walls of the spores. That this line, however, in reality represents from the start two closely appressed surfaces is abundantly shown in many cases.

[1] Hertwig: Die Zelle und die Gewebe, p. 187.

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These lines of cleavage are not meridional furrows which divide the cell symmetrically, but they intersect each other at varying angles, marking off the surfaee of the cell by a network of grooves, in which the meshes are of an irregular shape and of unequal dimensions (Fig. 14, E).

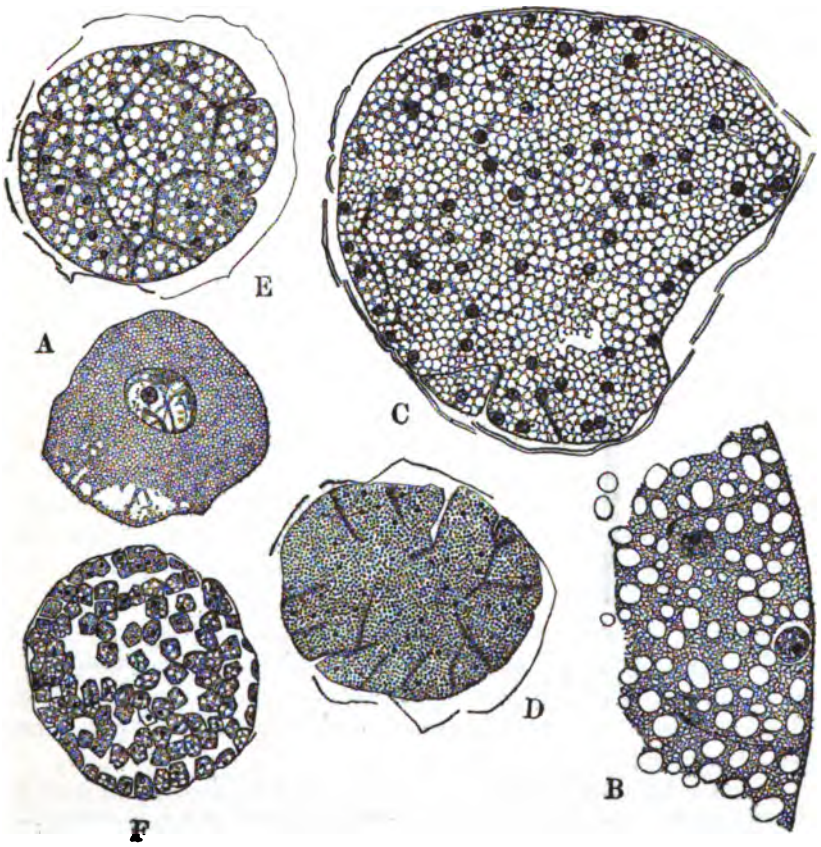

FIG. 14.—Cell-cleavage in *Synchitrium discipens*.

A, sporangium mother-cell.
B, Portion of cell showing two nuclei and two surface cleavage-furrows.
C, multinucleate stage, showing progressive cleavage by furrows from surface.
D, median section showing cleavage further advanced.
E, section from surface of cell in early stage of cleavage.
F, cell after segmentation is completed, showing uninucleate protospores.—(After Harper.)

The cleavage is progressive from the surface inward, the furrows deepening in general in a radial direction. Still they may be curved, and are inclined to each other at very varying angles and frequently form intersections at points near the surface of the cell, thus cutting off superficial blocks of protoplasm of varying shapes and sizes (Fig. 14, C), so that we have a central solid mass or

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cell of protoplasm surrounded by a layer of superficial cells; in other cases the furrows grow radially inward without intersecting till near the centre, thus forming narrow cones and pyramids with their bases outward (Fig. 14, D).

With the progress of cleavage the contraction of the protoplasm in *Synchitrium* becomes very noticeable, the furrows open widely and the masses tend to become rounded. The cell is thus split up into a number of blocks of varying size and containing a variable number of nuclei. In these large cells or portions of protoplasm cleavage furrows show no tendency to orient themselves with reference to the nuclei, but as the process advances and the pieces become smaller the nuclei are seen to be more evenly distributed. Finally, the result is always the separation of the cytoplasm into uninucleate masses or cells (Fig. 14, F).

It is interesting to note that the process which, in the beginning, seemed to be independent of the nuclei, is finally directed solely from the standpoint of their distribution.

From this process of cleavage in *Synchitrium* it is at once apparent that we have a method of cell-formation which is fundamentally different from either of the two methods described in the preceding pages. Here there are no kinoplasmic fibers developed in connection with the nuclei under whose instrumentality plasma membranes are formed, and, in earlier stages of cleavage in the sporangium, new plasma membranes seem to be developed independently of nuclei, though not in their absence.

In certain cases of cell-formation by cleavage, in which very large multinucleate masses of protoplasm are involved, as in the plasmodium of certain Myxomycetes and in sporangia of such Phycomycetes as *Pilobolus* and *Sporodinia*, vacuoles play a very important part either directly or indirectly.

The first indication of the cleavage which is preparatory to the formation of the columella-wall in the sporangium of *Pilobolus* (Harper, '99) is seen in the gradual appearance of a layer of vacuoles larger than the rest, and lying in the curved surface which marks the outline of the columella:

> The vacuoles become flattened in their radial axes parallel to the surface of the sporangium, and form thus disk-like openings which tend to fuse at their edges. At the same time a circular cleft is seen to start from the edge of the sporangiophore opening . . . and to develop upward, cutting into the vacuoles, so that they become connected into a continuous furrow (Fig. 15, A). Whether this furrow is continued upward to enclose the whole dome-shaped columella, or whether the vacuoles in the upper portion fuse edge to edge before the cleft reaches them, is difficult to determine. The process is a progressive one, the cleavage being complete in certain portions sooner than in others, and

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at a very late period strands of protoplasm are seen connecting the spore plasma with that in the columella. It is not impossible that many of the apparently disk-shaped vacuoles are sections of curved openings which burrow through the plasma from below upwards. Frequently vacuoles which are distinct in one plane are seen, by focussing up and down, to lie connected. There can be little doubt, however, that a considerable part of cleavage of the columella is accomplished by flattening and lateral fusion of originally ellipsoidal or spherical vacuoles; that is, the cleavage is not entirely by a furrow from the plasma

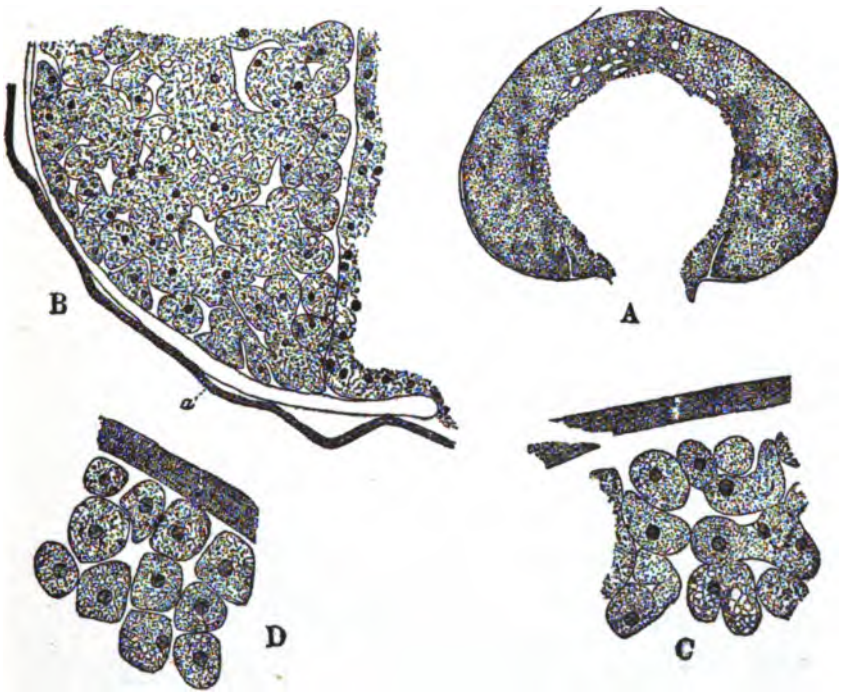

FIG. 15.—Cell-cleavage in sporangium of *Pilobolus crystallinus*.—(After Harper.)

A, median section at stage when columella is forming.
B, section of spore-plasma from base of sporangium, showing surface cleavage-furrows; *a*, sporangial wall.
C, section of portion of upper part of a sporangium, showing irregular sausage-shaped bodies formed by cleavage of spore-plasma.
D, similar to C, but older, showing uninucleate masses (protospores).

membrane at the mouth of the sporangiophore, but is at least in part a process of separation by excretion of a liquid into vacuoles and their fusion side by side *in situ*. These vacuoles are not situated on the extreme boundary of the protoplasm adjacent to the large central vacuole, but placed where the dense spore-plasma first becomes characteristically spongy. At the base of the sporangium indeed, they cut through plasma as dense as the densest spore-plasma of the sporangium. Why the cell-wall of the columella could not be deposited on the surface of the central vacuole, as well as on the surface of the small vacuoles,

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and thus enclose all the protoplasm in the sporangium, is an interesting question. The necessity is evident that the cleavage should proceed through a tolerably dense plasma, and this is, perhaps, due to the need of two protoplasmic surfaces in contact in order to form a cell-wall.

The fact that the columella is not deposited on the surface of the central vacuole seems to indicate that the limiting layer of a vacuole is not quite a plasma membrane, although it may partake partly of the real nature of one. Although there is much to show that the wall of a vacuole, such as we are dealing with here, and a plasma membrane are closely related, yet the author is not quite ready to admit that they are the same. Why two plasma membranes should be in contact in order to form a cell-wall, as suggested by Harper, is not quite clear to the author, since in many cases a single plasma membrane will secrete a cell-wall.

In the cleavage of the spore-plasma, which begins soon after the columella is complete, vacuoles also take an important part. The cytoplasm becomes somewhat vacuolar, and the numerous nuclei are rather evenly distributed throughout its mass. Cleavage furrows appear now near the base of the sporangium, cutting the surface into irregular polygonal areas (Fig. 15, B). At the same time vacuoles in the interior become angular, appearing three-cornered in section, and their edges cut through the cytoplasm to meet similar cleavage furrows from adjacent vacuoles (Fig. 15, B). In the meantime the surface furrows which have been growing deeper meet and become continuous with the edges of the vacuoles. By pressure of the adjacent plasma-masses, the surfaces of the vacuoles which were formerly convex become concave, and the vacuoles appear as intercellular spaces between the cleavage-segments. In this manner the spore-plasma is marked out into irregular blocks, apparently without reference to the size or number of nuclei they contain. A continuation of the process cuts the spore-plasma into oblong rounded sausage-shaped masses containing generally two to four nuclei in a row (Fig. 15, C). These oblong masses now divide transversely to form rounded bodies with one or few nuclei (Fig. 15, D). This completes the primary cleavage by which the spore-plasma has been cut up into smaller units with one or few nuclei. These units are not the spores. They undergo a period of growth and nuclear division before the final cleavage divisions take place by which the mature spores are produced. The last divisions are, however, similar to the first, presenting the simpler process of cleavage or fission.

In the sporangium of *Pilobolus*, we have a cleavage which is of the same type as in *Synchitrium*, with the exception of the promi-

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nent part taken by the vacuoles in the former. Although the membranes of these vacuoles may not, at first, be exactly similar to plasma membranes, they are undoubtedly converted into them. Since we assume that the plasma membrane is largely of a kinoplasmic nature, and attribute to it something of a morphological rank in the cell, it may not be wholly fanciful to suggest that the limiting membrane of a vacuole may be developed into a real plasma membrane, and that this actually takes place in the plants in question.

CELL-DIVISION IN DICTYOTA AND STYPOCAULON.

There is yet another method of cell-formation which has been observed in certain of the brown algæ that differs materially from the process of cleavage already described. There are no kinoplasmic connecting fibers by which a plasma membrane may be formed, nor is it a cleavage such as has been described for certain fungi.

The plasma membrane, or cell-plate, seems to be formed directly out of the apparently undifferentiated framework of the cytoplasm. This type of cell-formation has been observed in such *Phæophyceæ* as *Stypocaulon* (Swingle, '97), *Fucus* (Strasburger, '97), and *Dictyota* (Mottier, 1900).

Swingle has followed the development of the cell-plate in great detail in the apical cell of *Stypocaulon*. Here each division of the nucleus is followed by a cell-division. The bulk of the cytoplasm presents a very beautiful and typical alveolar structure, and the first indication of a cell-plate is seen in certain alveolæ, which show a tendency to arrange themselves across the cell in a transverse plane (Fig. 16, B). As soon as this orientation of the alveolæ becomes more marked, the transverse alveolar lamellæ form a more continuous plane which, in section, appears as a very fine line. During these changes neither an increase in the number of connecting fibers between the nuclei nor any perceptible change whatever in the arrangement of the kinoplasm was to be seen. Only a few fibers or lines of force, indicated by the arrangement of the alveolœ of the frothy plasma, extend from the nucleus of the apical cell to the seat of cell-plate formation, and fewer still from the lower nucleus to the same place. It is certain that if there be real fibers, they must be extremely delicate and not numerous enough to lead one to suppose that the cell-plate is laid down by any such process as in the higher plants.

The author has found that the development of the plasma membrane in the tetraspore mother-cell of *Dictyota* (Mottier, 1900) is similar to that of *Stypocaulon*. Here there is absolutely no visible trace of

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kinoplasmic connecting fibers between the nuclei, and in the region of the cell-plate the cytoplasm seems undifferentiated. The plasma membranes, or cell-plates, which will separate the four spores, are laid down almost simultaneously. In the region where they are to appear the cytoplasm, as elsewhere, except near the nuclei, presents the same visible structure of alveolæ, or perhaps a mixture of alveolæ and a thread-like network. Rather large and small meshes are intermingled.

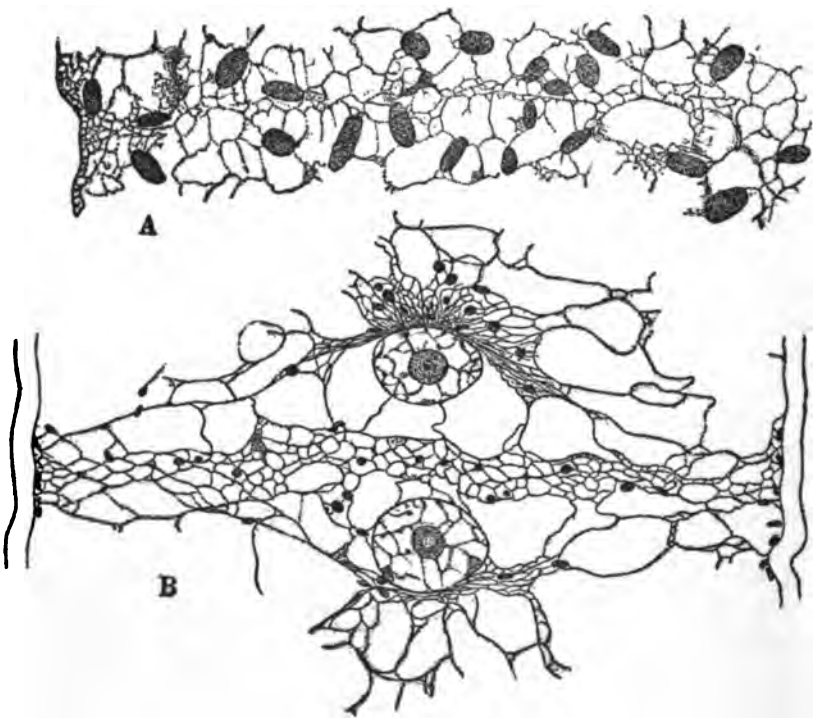

FIG. 16.—Cell-plate of *Dictyota dichotoma* and *Stypocaulon*.

A, portion of cell-plate from tetraspore mother-cell of *Dictyota*, formed apparently by arrangement of alveolar lamellæ into a continuous and even plane.
B, same from apical cell of *Stypocaulon*.—(B, after Swingle.)

The small-meshed structure is apparently more granular than that with larger meshes.

The first visible trace of a cell-plate is manifested by the transverse walls of the alveolæ becoming perceptibly thicker and arranging themselves in such a way as to appear as an uneven or somewhat zigzag line in section (Fig. 16, A). In this cell-plate primordium the walls of both large and small meshes take part. At first certain of the alveolar lamellæ are thinner than others, so that the cell-plate seems

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interrupted at these places, but eventually and gradually it attains a uniform thickness. Very soon the cell-plate is a uniform plane, appearing in section as a rather smooth line.

The cell-plate is not always laid down everywhere simultaneously, but sometimes it appears at first more marked at the periphery. This seems to depend upon the position of the nuclei. It is evident that in *Dictyota* no differentiated kinoplasmic connecting fibers can be recognized by which the cell-plates are formed. It seems that the apparently undifferentiated framework of the cytoplasm, consisting of large and small meshes in the immediate region of the cell-plate, is converted into a plasma membrane. The cell-plates are certainly formed under the influence of the nuclei, and kinoplasm in some form enters into the process.

The behavior of the cell-plate toward certain stains, particularly gentian violet, and the character and behavior of the cytoplasm in that region, immediately preceding the appearance of the plasma membrane, strongly suggests that the latter is not an actual transformation of the alveolar walls, but that the substance of the cell-plate is deposited by kinoplasm present in the framework of the cytoplasm. The form in which this kinoplasm occurs here is difficult to determine, but it matters very little whether it takes on the form of a fibrous network or of alveolæ, or whether it is present merely as a homogeneous fluid.

Of the several types of cell formation briefly described in the foregoing pages, the first, or that which is typical for higher plants, occurs generally in all plants from the liverworts up. It obtains also in *Chara* and *Nitella* and has been found by Fairchild ('97) in *Basidiobolus*. This method doubtless occurs in other algæ and fungi.

The process of typical free cell-formation, as found in the ascus of the *Ascomycetes* mentioned, is, so far as known, restricted to this group of fungi

A process of free cell-formation has been described by Strasburger in the egg-cell of *Ephedra*, but there it differs considerably from that in the ascus, since centrosomes or centrospheres are not present and the kinoplasmic fibers radiate in all directions from each nucleus.

The process of cleavage is the method of cell-formation in the plasmodium of *Myxomycetes* and in certain *Phycomycetes*. It is also of undoubted occurrence in many algæ and in other fungi.

Whether the kind of cell-plate formation described for *Stypocaulon* and *Dictyota* occurs outside of the brown algæ, future research must determine.

The process of constriction characteristic of *Cladophora* and *Spirogyra* may be looked upon as a kind of cleavage in which the formation

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of the new cell-wall is gradual and progressive from the old cell-wall inward, instead of being developed simultaneously from a plasma membrane previously formed. Whether in such cases new plasma membranes are formed across the ends of the daughter cells which come in contact with the new transverse cell-wall the author is unable to state.

THE CENTROSOME AND THE BLEPHAROPLAST.

As illustrations of karyokinesis in which the spindle arises through the agency of centrospheres I have selected the tetraspore mother-cell of *Dictyota* and the ascus of certain *Ascomycetes*, because the centrosphere is probably best known in those cells and because the entire development of the mitotic figure has been followed in great detail. In these plants, as well as in *Fucus* and certain *Sphacelariaceæ*, we have seen that the body which we call a centrosome is one that persists from one cell-generation, or nuclear generation, to another in vegetative and in certain reproductive cells. It seems to be capable of division, and is the centre of radiations that give rise to the karyokinetic spindle. We do not know with absolute certainty that the centrosome divides, although the evidence seems to admit of no other interpretation.

In addition to the plants just mentioned, centrospheres have been found in some liverworts, in diatoms, and in certain *Rhodophyceæ*. In the diatoms, however, the behavior of the centrosome during karyokinesis, as described by Lauterborn ('96), differs widely from the typical cases described in the preceding pages. In species of *Pinnularia*, *Surirella*, and others, Lauterborn finds that the peculiar central spindle arises from the centrosome by a division or process of budding. "Es scheint mir keinem Zweifel zu unterliegen, dass die Anlage der Centralspindel aus dem Centrosom durch eine Theilung (oder, wenn man lieber will, Knospung) hervorgeht" (l. c., p. 61).

In the diatoms in question the original centrosome is a relatively large globular body which is the center of a system of beautiful radiations. Soon after the budding off of the primordium of the central spindle, the original centrosome, with its radiations, disappears, and what is taken to be the new centrosomes arise near the poles of the spindle and apparently from it.

So far as the author is aware, such a phenomenon has no parallel among plants, and it is impossible to bring the process of spindle-formation in the diatoms, as described by Lauterborn, into line with anything known in other organisms.

When we consider the facts alone in the algæ and fungi mentioned, we certainly have strong evidence in favor of the doctrine of the genetic

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continuity of the centrosomes; but from the fact that no such organs exist in the higher plants, and that they seem to be wanting in many *Thallophyta* as well, this view is greatly weakened, if not rendered quite untenable.

On the zoological side of the question, the recent researches of Wilson (1901) on eggs of *Toxopenustes*, which were made to develop parthenogenetically through certain stages by means of chemical stimuli, throw new light upon the subject. In segmenting eggs induced to develop parthenogenetically by means of a treatment with suitable solutions of magnesium chloride, numerous asters (cytasters) often made their appearance in the cytoplasm in addition to the nuclear asters. Similar asters may arise also in non-nucleated fragments of eggs. These "cytasters," just as the segmentation or nuclear asters, may consist of a very distinct centrosome upon which is centered a system of beautiful radiations. The centrosomes divide, and a central spindle is formed between the daughter centrosomes. In fact, the "cytasters" are exactly like the normal cleavage-asters arising in connection with the chromatin. As the evidence seems conclusive that the "cytasters" arise *de novo*, Wilson concludes that centrosomes occurring normally in cells arise also *de novo*, and that the doctrine of the genetic continuity of the centrosome is untenable.

It is not known whether anything comparable to these "cytasters" ever occurs in a plant egg-cell, which may be made to develop parthenogenetically by artificial means, and consequently we cannot accept the conclusion upon this basis as applicable to plants. There are, however, in plants many well established facts which argue strongly against the view that the centrosome or centrosphere is an organ of morphological rank.

In 1897, the author made the unqualified statement, to which he still adheres, that centrosomes or centrospheres do not occur in the higher plants, and nearly all research since made along this line has only confirmed this view. We know now that the structures which Guignard so beautifully figured in 1891 for cells of *Lilium* were the product of preconceived ideas and the misinterpretation of certain facts. There are still a few observers who persist in seeing centrospheres in the cells of higher plants, in which a score or more of the most competent cytologists, with the aid of the very best methods, have failed to find any such structures. It may be of some interest to note, however, that the centrospheres figured more recently by these observers are not drawn with the old-time diagrammatic distinctness, and it will probably not be long till these structures will not appear at all in figures illustrating karyokinetic phenomena in *Allium cepa* and species of *Lilium*.

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At the present writing it is the opinion of the author that individualized centrosomes or centrospheres do not occur in plants above the liverworts, and they are certainly absent in certain species of these (*Anthoceros*). On the whole, these structures are well established in only a few Thallophyta.

As the writer has already stated in a former paper (Mottier, 1900), if we take into consideration only such plants as *Fucus*, *Stypocaulon*, *Dictyota*, and certain *Ascomycetes*, there are good grounds for the view that the centrosome is an organ of morphological value; but the evidence furnished by these forms, however convincing it may seem, is not quite sufficient, especially in the light of our knowledge of karyokinesis in forms in which centrosomes or centrospheres have not been found; for there is no reason for believing that the spindle fibers in plants devoid of centrosomes are of a different substance from the radiations or spindle fibers developed in connection with an aster.

Space will not permit of a discussion of such questions as whether the radiations are outgrowths of the centrosome considered as a morphological unit, or constructed out of the kinoplasm by the centrosome, or whether the centrosome is only a denser mass of kinoplasm, formed by the meeting of the polar radiations, and which may persist after the radiations and spindle fibers have disappeared. It may be stated in this connection that in plants there is little to support the view that the radiations are centripetal or centrifugal currents. They do not seem to be currents at all. We understand radiations and spindle fibers to be fine, more or less homogeneous, kinoplasmic threads which are capable of contracting, extending, or becoming changed into a uniform and homogeneous mass.

We have now to consider the relation of the centrosome to the *blepharoplast*, or cilia-bearer, which is so well known in the spermatozoid of the *Archegoniates* (see Chapter V).

Belajeff, Ikeno, and Hirase and a few others regard the blepharoplast of the fern and certain gymnosperms as the homolog of the centrosome. It seems to the author that such a conclusion is merely a hasty judgment, which does violence to the facts as they are known at present. The development and function of the blepharoplast, as will be seen from the chapter referred to, shows clearly that this structure lacks the more essential distinguishing characteristics of the normal centrosphere, as it is known in the cases most thoroughly investigated. The blepharoplast is not the center of kinoplasmic radiations which form a karyokinetic spindle. So far as has been shown the radiations of the blepharoplast primordia take no part in the formation of the spindle. These primordia do not divide to give rise to new blepharoplasts, but

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arise *de novo*. They do not persist through several successive generations of cells, two cell-generations representing the maximum time of their duration. In short, the blepharoplast develops merely the cilia and forms, therefore, the locomotary apparatus of the spermatozoid.

No phylogenetic relationship has as yet been shown to exist between blepharoplast and centrosome. The fact is that, in those plants in which blepharoplasts occur, there are no centrosomes with which to show any phylogenetic relationship. The main reason, it seems, for regarding the blepharoplast as the homolog of the centrosome is the sole fact that the primordia of the former at a certain period of development are provided with a system of radiations, giving them the appearance of centrospheres.

The view concerning the origin and phylogeny of the blepharoplast as advanced by Strasburger is of interest, since it is the only one that seems to take into consideration all the facts. Strasburger derives the blepharoplast from the cilia-bearer of the zoöspores and gametes in the algæ. In the zoöspores of certain algæ, *e. g.*, *Vaucheria*, *Œdogonium*, and others, the cilia spring from a localized thickening of the plasma membrane (Hautschicht) at the anterior end. In *Œdogonium* this kinoplasmic thickening is in the shape of a doublc convex lens, from the edges of which arise the numerous cilia. In the large swarm spore of Vaucheria the nuclei seem to be intimately connected with the formation of the cilia-bearer. The nuclei migrate to the plasma membrane and elongate in a direction at right angles to the surface of the spore. The anterior end of each pear-shaped nucleus comes in contact with the plasma membrane. That part of the plasma membrane in contact with the nucleus thickens in the form of a delicate concavo-convex lens, from two points of which, on opposite sides, spring the cilia. The size and shape of the cilia-bearer vary, of course, in different algæ. Timberlake ('01) finds a small body at the base of the cilia in *Hydrodictyon*, but it does not seem to be part of the plasma membrane.

As Strasburger has pointed out, the " mouth-piece " of swarm spores and gametes is not to be confounded with the cilia-bearer, since the former represents the entire anterior end of the cell free from chlorophyll. It is true that the cilia-bearer is not well known in the spermatozoids of algæ, but transitions show that in all probability the spermatozoids were derived from male gametes which in every way resembled asexual swarm spores. The spermatozoids of *Volvox globator* are regarded as a good illustration of this relation, for in structure they occupy an intermediate position between the gametes of algæ and the spermatozoids of *Chara*. In *Volvox* the two laterally inserted cilia would seem to indicate that the blepharoplast had undergone a lateral

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displacement, for the entire anterior end of the spermatozoid of *Volvox* is certainly not blepharoplast. (The very suggestive theory of Strasburger carries with it a certain degree of probability, yet to what extent it is true further research must determine.)

If, however, any genetic relationship exists between centrosome and blepharoplast, the evidence is certainly to be sought in the lower plants. In this connection it is of the greatest importance to know first of all whether, in such algæ as the *Sphacelariaceæ*, in which centrosomes are known, any relation exists between the centrosome and cilia-bearer, assuming, of course, that the cilia arise here also from a differentiated body. In *Chara* and in those *Archegoniates*[1] with blepharoplasts no centrosomes are found, neither is any such body known to take part in the formation of the spindle in such algæ as *Œdogonium*, and others in which highly developed cilia-bearers occur. Although these facts do not prove anything, yet they lend encouragement to the belief that centrosome and blepharoplast may be homologous structures, or in some degree phylogenetically related.

Those who maintain that the cilia-bearers are centrosomes have not, it seems, approached the question from the standpoint just mentioned, but seem to have based their conclusion upon the resemblance between blepharoplast primordia and centrospheres, or upon analogies between the spermatozoids in plants and the spermatozoa of certain animals.

Belajeff ('99), who claims that blepharoplasts are homologous with centrosomes, strengthens his view by his observations in spermagenous cells of *Marsilia*. In the grandmother-cells of the spermatozoids of this plant he finds that the blepharoplast primordia, which lie some distance from the nucleus, divide previous to the division of the nucleus, and between the two separating daughter primordia a small central spindle is developed just as in certain animal cells. From this small amphiaster the karyokinetic figure is developed. This, if true, is the first case on record in plants in which a central spindle is formed between the daughter centrosomes, lying in the cytoplasm some distance removed from the nucleus.

In the light of what is now known concerning the development of the spindle in *Chara* and in the *Pteridophyta*, the author entertains serious doubts concerning the accuracy of Belajeff's statement. Osterhout's ('97) studies on the development of the spindle in the spore mother-cells of *Equisetum* prove beyond all question that centrosomes are not present in that genus. In other *Pteridophyta* the majority of all investigations, which have been thorough or reasonably exhaustive, shows that centrosomes or centrospheres are absent there also.

[1] *Marchantia polymorpha* excepted.

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From our present state of knowledge of the development of the blepharoplast there is but one conclusion, it seems to the author, that can be legitimately drawn concerning their origin, namely, that they arise *de novo*. As regards centrosomes the evidence is more complicated and conflicting. Although, in the opinion of the author, the evidence is decidedly against the doctrine of the genetic continuity of the centrosome, yet the proof is not quite conclusive. If centrosomes also arise *de novo*, then the problem assumes a slightly different aspect, for it is questionable whether we are justified in speaking of homologies between organs that, as such, are without genetic continuity.

There is strong evidence, which seems to be increasing from day to day, that it is the fundamental substance known in the plant cell as kinoplasm which is genetically continuous. After a careful consideration of the facts, the author is led to the same conclusion concerning the centrosome to which he gave expression in 1900, in a paper on the nuclear division in *Dictyota* (l. c., p. 178), namely, that it is the kinoplasm which should hold the rank of morphological unit, and that the centrosome should be regarded as an individualized part of the same, existing in that form in some organisms and not in others, for reasons that cannot at present be explained. As regards blepharoplasts, about the only conclusion in harmony with all the facts is that these bodies represent individualized parts of the kinoplasm which arise *de novo* in certain spermagenous cells, and from which the cilia are developed.

SIGNIFICANCE OF THE SEXUAL PROCESS AND THE NUMERICAL REDUCTION OF THE CHROMOSOMES.

Speaking generally, the phenomena resulting from the sexual process fall into two categories, namely, (1) the transmission of hereditary characters, together with the blending of two lines of descent by the fusion of the sexual nuclei, and (2) the imparting of a growth stimulus to the fecundated egg or to the zygote, by which the energy of growth and division is restored.

Correlative with the first category is the reduction in the number of chromosomes. The doctrine of the significance of the numerical reduction of the chromosomes now generally accepted by botanists as a working hypothesis, was first stated in a well organized form and presented formally to botanical science by Strasburger ('94) in a masterly essay on the "Periodic Reduction of Chromosomes in Living Organisms." The enunciation of this doctrine marked the beginning of a new epoch in the study of sexuality and in cytological research in plants.

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The simplest and most primitive organisms known reproduce themselves asexually, and we are obliged to assume that, from a phylogenetic standpoint, sexually differentiated organisms were descended from asexual forms. The process of this descent is clearly illustrated by certain of the green algæ in which the sexual act consists in the fusion of exactly similar motile gametes. These gametes were undoubtedly derived from asexual swarm spores, which they closely resemble, except in that they are smaller and often have fewer cilia. In *Ulothrix*, for example, and in many of the green algæ, the gametes are, so far as is known, smaller and possess only two cilia, while the larger asexual swarm spores bear four cilia. Both sporangia and gametangia are homologous structures, and, so far as is known, the gametes differ only physiologically from the asexual spores.

According to Strasburger, to use the language of the translation :[1]

The sexually differentiated plants manifest certain differences in their ontogeny, from which it is possible to infer what was the course along which the phylogenetic differentiation proceeded after sexual differentiation had taken place. The simplest case is that in which the product of fertilization gives rise to an individual similar to those which gave rise to the product of fertilization, and which closes its own life history with the development either of sexual organs or of asexual organs homologous with them. This occurs in many *Chlorophyceæ*, where, from the zygospore (the product of the coalescence of similar gametes) or the oöspore (the product of the coalescence of dissimilar spermatozoids and ova), a generation is developed which resembles the preceding and gives rise either to swarm-spores or to sexual cells homologous with them. Generally, any one sexual generation follows after a number of asexual generations, the relation being, however, dependent on external conditions, so that, as Klebs has shown, the development of a sexual or an asexual generation can be determined by the observer. In such cases there is a homogeneous sequence of generations which does not include any other kind of sequence or alternation beyond the development either of asexual reproductive organs or of sexual organs homologous with them. The asexual reproductive organs are especially concerned with the rapid multiplication of individuals under favorable external conditions; whilst sexual reproduction is of importance in maintaining the existence of the species under circumstances which are unfavorable to the vegetative existence of the individual. At the same time, sexual reproduction ensures certain advantages arising from the coalescence of distinct sexual cells.

In proportion as the asexual mode of reproduction was replaced by the sexual, the numerical conditions of multiplication were maintained either by the development of a number of oöspores, as in certain Fucaceæ; or, in addition to the sexual organs, altogether new organs were developed to ensure rapid and vigorous development of new individuals in an asexual manner. This took place in various ways. Either asexual reproductive organs were intercalated in the life history of the original generation, or an altogether new asexual generation was developed from the product of the sexual act.

[1] English translation, Ann. Bot., 8 : 281–316.

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I have quoted thus at length because it seems that this statement of Strasburger is a compact and concise summing up of the phylogenetic development of the process of reproduction and multiplication of individuals among the lower plants.

The intercalation of new asexual reproductive organs into the original generation is strikingly illustrated in many of the fungi, in which the independent individualization of the different stages of development of the sexual generation into special organs of vegetative multiplication, or even into distinct individuals, was carried so far that sexuality seems to have disappeared entirely, as in the higher fungi. On the other hand, in all plants beyond and including the Bryophyta there arose an altogether new generation as the product of the sexual act, whose function is to produce asexually a large number of individuals. The degree of development attained by the new generation in the plants above the Thallophyta differs according to whether its activity was limited to the production of asexual spores alone, or included nutritive functions as well, or whether it became an independent individual. In the *Bryophyta*, especially in some of the simpler liverworts, the new asexual generation is confined almost exclusively to the production of spores, *i. e.*, to the multiplication of the individual, while the original or sexual generation upon which all nutritive function is devolved, together with vegetative multiplication as well, has attained in many cases a cormophytic differentiation. In the *Pteridophyta* and in the higher plants, on the contrary, the center of gravity of phylogenetic evolution is transferred to the new or asexual generation arising from the act of fecundation, and in these plants the asexual generation has attained its highest cormophytic development. Among the *Pteridophyta* of the present time it is evident that (l. c., p. 283) "as this evolution took place, the nutritive apparatus of the sexual generation became of less importance, and it became altogether superfluous from the moment when the asexual generation began to provide its spores with the material necessary for the development of the sexual generation." Along with this evolution there came into existence, as a correlative phylogenetic process, the dimorphic character of the gametophyte, which is characteristic of certain *Pteridophyta* and of all *Spermatophyta*. This dimorphism was probably manifested in the character of the mature gametophyte before any visible trace of it could be recognized in the unicellular stage of the sexual generation, namely, the spore. To illustrate this fact we need only to recall the condition which obtains among certain homosporous *Filicineæ*, for example, *Onoclea struthiopteris* of the *Polypodiaceæ*. Here there is no visible evidence of heterospory, yet it is perfectly well known that in every

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culture of spores some will develop into distinctively male prothallia, bearing only antheridia, while others show a marked tendency to develop into prothallia bearing only archegonia. It is also well known that this tendency toward dimorphism is, in a measure, influenced by external conditions, for if spores of *Onoclea struthiopteris* be sown thickly, and the culture be poorly illuminated and, consequently, poorly nourished, the vast majority of the prothallia will be male; but if the spores be sown thinly and well illuminated, a much greater number will become female plants.

In all existing forms in which the spores, or unicellular condition of the sexual generation, contain food material for the development of the asexual generation, or its earlier stages, dimorphism is well established, *i. e.*, those forms are heterosporous, and the conclusion which most naturally follows is that heterospory and the disappearance of the nutritive apparatus of the sexual generation represent correlative phylogenetic processes.

Now, during this phylogenetic evolution and, as Strasburger very clearly puts it,—

> In accordance with the general law which determines the phylogenetic disappearance of organs which have become useless, the vegetative parts of the sexual generation became more and more reduced, until little was left but the reproductive organs themselves: hence the progressive reduction in the prothallium from the Ferns up to the Phanerogams. This reduction culminated in the complete loss of independent existence by the sexual generation, because it had ceased to be able to nourish itself independently, and [because of] its becoming enclosed by the asexual generation. In consequence of this enclosure of the sexual in the asexual generation, the advantageous rapid multiplication of individuals which the latter originally effected was lost: in order to compensate for this loss, a large number of seeds were produced in the Phanerogams in place of the numerous spores of the Cryptogams; that is, multiplication is effected now by the product of fertilization instead of by asexual spores.

In harmony with this doctrine, an alternation of generations is necessary in those plants in which the fecundated egg gives rise to the asexual generation, and the asexual spore to the sexual generation.

The development of the plant kingdom, at least so far as sexuality is concerned, seems to show that sexual differentiation was preceded by asexuality, and in those groups in which a true alternation of generations exists the sexual generation is to be regarded as the older and more primitive and as having arisen from an asexual form. In fact, we are able to trace this phylogenetic development step by step, or the evidence at hand, at least, seems to be sufficiently conclusive to justify the general acceptance of the doctrine. Probably the first indication of this development is to be found among such algæ as *Œdo-*

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gonium, *Coleochæte* and, as the researches of Oltmanns seem to indicate (See Chapter IV), certain *Rhodophyceæ*. From the fecundated egg of *Œdogonium* four swarm-spores are developed, while in *Coleochæte* a multicellular body is developed, from the cells of which asexual swarm-spores are formed. In both cases the swarm-spores give rise to sexual plants, or the first generation. The product of the fecundated egg in *Coleochæte* bears a striking resemblance to the sporophyte of such liverworts as *Riccia*. The fundamental differences lie chiefly in the fact that the covering of the sporophyte in *Coleochæte* is derived from vegetative branches of the thallus, the oögonium being unicellular, and that the asexual spores are motile, a correlation with the aquatic habit of *Coleochæte*. In the *Rhodophyceæ* the cystocarp or cystocarps are the product of the fecundated egg, and the spores give rise to the first generation. This is made all the more probable by the researches of Oltmanns, which go to show that the fusion of the cells of the sporogenous filaments with auxiliary cells is merely a nutritive process. It is of interest to note further that a similar condition is preserved in certain *Ascomycetes* in which Harper has proved that *unquestioned* sexuality exists. Such algæ as *Coleochæte*, therefore, seem to point out more or less clearly the phylogenetic road along which the ancestors of the Archegoniates have passed.

Research upon the process of fecundation and indirect nuclear division, especially in reproductive cells, during the past twenty years, has given a new insight into the significance of sexuality and the alternation of generations in plants. Our knowledge along this line was very materially advanced by the discovery of Van Beneden ('83) that the number of chromosomes is the same in both conjugating nuclei. Further investigations have established the still more important fact that, in both plants and animals, a reduction to one-half of the number of chromosomes in the sexual nuclei preceded the sexual act, and that, as a consequence of the fusion of the male and female nuclei, the number of chromosomes in the fecundated egg is doubled.

In all the higher plants it is a well-established fact that the numerical reduction of the chromosomes takes place in the spore mother-cell, and that in the cells of the gametophyte arising from the spore the reduced number persists. In cells of the sporophyte, resulting from the fecundated egg, the increased number obtains until the differentiation of the spore mother-cells. It will thus be seen that the fundamental characteristic of both sexual and asexual generations lies in the number of the chromosomes, and upon this phenomenon rests the sexual differentiation of cells.

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There is a possibility that this doctrine may not be applicable to cases of apogamy, apospory, and normal parthenogenesis among plants. It has been suggested by Strasburger ('94, p. 300) that the number of chromosomes may become doubled under the influence of correlative processes in an apogamously developed fern which arises as a bud from the prothallium, the nuclei of whose cells contain the reduced number, and for the same reason the reverse may take place in cases of apospory, *i. e.*, the aposporous development of prothallia may be attended with a correlative reduction in the number of chromosomes. Until the facts are determined by actual observation, all discussion of this subject must remain a matter of pure speculation.

The researches of Juel (1900) upon the normal parthenogenesis of *Antennaria alpina* are of the highest interest in this connection, as they throw light upon this question so far, at least, as the seed-bearing plants are concerned. In *Antennaria alpina*, in which the egg develops parthenogenetically under normal conditions, Juel finds that no reduction in the number of chromosomes takes place in the development of the embryo-sac, and, consequently, the nucleus of the egg-cell which gives rise to the parthenogenetic embryo contains the same number of chromosomes as the vegetative cells. Contrary to *Antennaria dioica*, in which fecundation regularly occurs, the mother-cell of the embryo-sac of *A. alpina* develops immediately into the embryo-sac, the heterotypic and homotypic nuclear divisions which follow the appearance of the reduced number of chromosomes being omitted.

In cases of normal parthenogenesis among the angiosperms, the facts, so far as they are known, are certainly not at variance with the doctrine of the reduction of the chromosomes as applied to the alternation of generations.

As has been intimated in preceding paragraphs, the sexual generation has been spoken of as the more primitive condition, and, as will be seen from the following, the reduction in the number of chromosomes in the spore mother-cell is regarded by Strasburger as the return of highly organized plants to the original unicellular condition:

> The morphological cause of the reduction in the number of chromosomes and of their equality in number in the sexual cells is, in my opinion, phylogenetic. I look upon these facts as indicating a return to the original generation from which, after it had attained sexual differentiation, offspring was developed having a double number of chromosomes. Thus the reduction by one-half of the number of the chromosomes in the sexual cells is not the outcome of a gradually evolved process of reduction, but rather it is the reappearance of the primitive number of chromosomes as it existed in the nuclei of the generation in which sexual differentiation first took place (l. c., p. 288).
>
> The phenomenon under consideration is essentially that of the return of the

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most highly organized plants, at the close of their life-cycle, to the unicellular condition: in a word it is the repetition of phylogeny in ontogeny (l. c., p. 311).

This theory of reduction must still be regarded as a very helpful working hypothesis, finding its greatest application in the higher plants. In the lower cryptogams the theory is confronted with facts, many of which seem at present to be quite at variance with it. The product of fecundation in the *Thallophyta* as a rule does not give rise to a definite organism representing the asexual generation, and it is not known at which point in the life-cycle that reduction takes place. It has been suggested that reduction may take place during the germination of the zygote or oöspore. Conclusions respecting the time of reduction in the lower cryptogams have been drawn chiefly from the phenomena of certain cell-divisions that seem to be analogous with divisions which follow the reduction in higher organisms, and not from an actual determination of the number of chromosomes. On account of the many difficulties in counting, the number of chromosomes is known in only a very few algæ and fungi, and our knowledge on this subject is so meager with respect to these plants that the few definite facts that have been obtained, although apparently at variance with the theory, may not as yet be considered as offering very serious objections to it.

If the reduction in the number of chromosomes signifies what is attributed to it by the theory, it is possible, in the light of facts that have been observed in such algæ as *Fucus* and *Dictyota*, that what is considered the sexual generation in the Thallophyta may not be homologous with the gametophyte of higher plants, assuming that homology is based upon the number of chromosomes. Farmer and Williams ('96, '98), and Strasburger ('97) have found that the reduced number of chromosomes in *Fucus* appears in the oögonium, while in vegetative cells of the thallus twice that number is present. Strasburger finds that in the first nuclear division in the oögonium the reduced number appears, fourteen to sixteen having been counted, and this number persists throughout the two succeeding mitoses. In vegetative cells of the thallus, which is regarded as the gametophyte, the number is not far from thirty. In *Dictyota* I have found the reduced number (sixteen) of chromosomes in the first nuclear division of the tetraspore mother-cell, while in the vegetative cells of the thallus bearing the tetrasporangia about twice that number was counted. Whether in the nuclei of plants arising from tetraspores the reduced

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number persists, and whether in the egg-cell this number obtains was not determined.[1]

As is well known, two views are held concerning the manner in which the reduction in the number of chromosomes is accomplished. One of these views, which has been given prominence by Weismann, holds that the chromosomes are qualitatively different, and that reduction is accomplished during the maturation divisions in animal cells and in the first two divisions taking place in the spore mother-cells of higher plants. For example, in the second maturation division of the animal egg it is maintained that the daughter chromosomes do not arise as a result of a longitudinal splitting, but by a transverse division, or what is known as a qualitative division. The nuclei of the four cells thus resulting, whether representing the egg and its polar bodies or those which develop directly into spermatozoa, are hereditarily different in character, and it is upon this assumption that hereditary variation is based.

The other view, which is now very generally accepted by botanists, is that, in plants no qualitative division exists, but the chromosomes of each mitosis arise in every case by a longitudinal splitting. The reduction takes place in the resting nucleus or during the early prophase of the first, or heterotypic, mitosis in the spore mother-cell of higher plants. The fact, as shown in preceding paragraphs, that during this first mitosis a double longitudinal splitting of the chromosomes occurs, probably as a preparation for the two divisions, has led to much confusion, because these divisions were supposed to have been rather the instrument of reduction than a consequence of reduction.

Assuming the persistent individuality of the chromosomes, we may conclude on good grounds that the reduction represents the actual and complete fusion of the chromosomes of both parents, which have remained separate in the sporophyte until the formation of the spore mother-cells. There is no visible evidence that a qualitative difference exists between the chromosomes in plants, and our assumption here is that they are hereditarily similar, because of the fact that every indirect nuclear division is preceded by a longitudinal splitting of the chromatin.

Since the nucleus is the unquestionable bearer of hereditary characters, fusion of sexual nuclei in fecundation has for its purpose the blending of two lines of descent and possibly the restoration

[1] J. Lloyd Williams in a recent paper (Studies in the Dictyotaceæ, Ann. Bot., 18: 141–160, 1904) observes facts that seem to point to the conclusion that the plantlets developing from the tetraspores, with their reduced number of chromosomes, may become gametophytes, and that the fecundated egg cells probably develop into tetraspore plants which have been shown to possess the increased number of chromosomes. If this be true, an alternation of generations exists in *Dictyota*.

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of the power of growth and cell-division. The influence of the hereditary characters of each parent upon each other by their intimate association in the same nucleus seems to be the physical basis of phylogenetic variation, but the manner in which this influence acts to bring about variation, or to impart a more vigorous character to the product of fecundation still remains a matter of speculation.

It is well to consider the blending of the two lines of descent as a consequence of fecundation in a relative sense or as a correlative phylogenetic process. In certain of the lower cryptogams, *Ulothrix* and *Basidiobolus* for example, in which the gametes arise from adjacent cells of the same filament and in which a sexual differentiation is not at all or only scarcely recognizable, there does not seem to be two lines of descent to blend, yet it is conceivable that the sexual character of the nuclei may have been determined before the stage of ontogeny is reached in which the sexual cells manifest themselves as such. If in such forms a reduction in the number of chromosomes occurs, the sexual character of the nuclei is determined at that time. It is well known that among the simpler forms of the algæ and fungi, the development of gametes depends to a certain extent upon external conditions, which effect transpiration, atmospheric pressure, food supply, and so forth, yet no one would suppose for one moment that sexuality is the outcome of these external conditions.

We have now to touch briefly upon the category of phenomena by which a growth stimulus, or the power of growth and cell-division, is imparted to the product of fecundation. Among many of the lower algæ about the only important difference which seems to exist between a gamete and an asexual swarm-spore is the ability of the latter to develop into a normal individual of the adult size. It is true that the iso-gametes of algæ, such as *Ulothrix*, are capable of developing into small dwarf individuals—a fact which indicates that here, at least, the gametes possess the power of independent growth sufficiently to enable the resulting plantlet to develop to a limited extent. As soon, however, as the sexual elements have attained any marked degree of bisexual differentiation in the plant kingdom, the individual gametes are quite incapable of independent development even into the most rudimentary individuals, cases of normal and artificial parthenogenesis excepted.

The stimulus to growth and division in bisexual reproductive cells is imparted normally only by the fusion of male and female elements, and the question naturally arises, is this stimulus due to the fusion of the cytoplasm of the male cell with that of the female, or is it due merely to the fusion of the respective nuclei? Experiments upon arti-

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ficial parthenogenesis, brought about by the use of chemicals and other stimuli, have thrown some light upon the subject, but in the opinion of the author they are, as yet, far from furnishing an adequate solution of the problem.

In *Marsilia vestita* Nathansohn (1900) found that it was possible to stimulate the egg-cell to a parthenogenetic development by exposing the germinating macrospores to a temperature of 35° C. for 24 hours, and allowing them to continue their development at a temperature of 27° C. As a result about 7 per cent. of the spores gave rise to parthenogenetic embryos. So far as we know, this is the only case among plants above the *Thallophyta* in which parthenogenesis has been brought about artificially, and it may be that *Marsilia* lends itself to this sort of experiment more readily because of the fact that in certain species the tendency toward normal parthenogenesis is strongly manifested. In *Marsilia drummondii* Shaw ('97) found normal parthenogenesis to be of frequent occurrence. In these cases of *Marsilia* the morphological side of the question, especially the behavior of the nucleus, is not known, nor have the number of chromosomes been determined in the cells of the parthenogenetic embryo.

On the animal side of the question the experimenter finds, fortunately, an abundance of most favorable material in the eggs of sea-urchins and of certain marine worms. The results of several investigators (Wilson, Morgan, Loeb, and others) have shown that the eggs of *Arbacia* and *Toxopenustes* may be made to develop parthenogenetically through certain earlier stages by subjecting them for a certain time to a solution of sea-water, whose osmotic power is increased by the addition of a solution of magnesium chloride. The action of the Mg-solution seems to be similar to the growth stimulus imparted to the egg by a spermatozoön in normal fecundation.

Equally instructive are the experiments of Winkler (1901) on nucleated and enucleated fragments of the egg of *Cystosira barbata*, one of the *Fucaceæ*, which were fecundated by the spermatozoids. Both the enucleated fragments and those containing the nuclei developed into small embryo plantlets which were exactly alike and attained about the same stage of development.

The development of normally fecundated fragments of egg-cells and that of the entire eggs induced to develop parthenogenetically by chemical or physical stimuli are phenomena which seem to fall into the same category. They show that in all probability the growth stimulus, or the restoration of the power of division and the blending of hereditary characters are phenomena which in a measure are independent of each other. Experiments similar to the foregoing have

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their greatest value in the suggestiveness of their results and the new points of view to which these results lead. They do not show that the reactions brought about by these stimuli are the same as those resulting from the union of sexual cells. Although the development of a rudimentary embryo induced by artificial means may proceed in the same manner as the product of normal fecundation, yet the artificial stimulus cannot be looked upon as being equivalent to the sexual process. In the case of the former, we are dealing with a stimulus which merely starts growth, but a mature individual is never developed. The sting of an insect or some similar stimulus may call forth a growth in a leaf of an oak, which results in a gall, a local and limited growth, but never in an oak tree, and we cannot for one moment think of comparing such a stimulus to a sexual process.

The author does not agree with those who regard the sexual process merely as a restoration to the egg of the power of growth and division. We are not quite ready to lay aside, as yet, the facts won by twenty years of the most careful morphological research for any chemical or electrical theory of heredity.

Our knowledge of sexual reproduction in the plant kingdom indicates beyond question that that which is of primary significance in the sexual process is the fusion of the nuclei, and the question still remains, which imparts the growth stimulus, the nucleus or the cytoplasm of the sperm? Or are both necessary?

Strasburger has suggested that the stimulus to growth and division is given by the cytoplasm, and especially a particular part of the same, the kinoplasm, brought into the egg by the spermatozoid. Some zoölogists have attributed this stimulus to the centrosome of the sperm, but in the plant kingdom no case is definitely known in which a centrosome is brought into the egg by a spermatozoid. The doctrine of Strasburger is perhaps the best that has been proposed, and it seems to have some basis in fact. According to this view the egg is rich in food material, trophoplasm, and poor in kinoplasm, while in the sperm the reverse obtains. The unfecundated egg is incapable of developing, therefore, on account of the lack of energy.

This theory, however plausible it may seem, leaves much to be desired. In the first place, it is not known as a fact that the egg is poor in kinoplasm, and that the sperm is correspondingly rich in that substance. In many cases the quantity of cytoplasm of the male cell is so small that it seems almost incredible that it could have such a powerful influence. The spermatozoid of the fern, for example, consists of a relatively very small amount of cytoplasm, and the kinoplasmic part of this constitutes an organ of locomotion. Although

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cytoplasmic band and blepharoplast, or cilia-bearer, enter the egg, yet their function seems to be of secondary importance as compared with that of the nucleus. Again in the higher seed-bearing plants, the generative nuclei are accompanied by only a small portion of cytoplasm, which cannot be recognized in the embryo-sac, and it seems reasonable that it is merely absorbed as so much food. However, when we remember that in all cases of fecundation at least some cytoplasm accompanies the male nucleus into the egg, there is good ground for the belief that the cytoplasm plays some important rôle, but whether that be anything more than to assist in restoring the power of growth and division must at present remain a question.

The behavior of the sexual nuclei during the process of fecundation and the wonderful phenomena of karyokinesis point to the conclusion that the nucleus is the bearer of hereditary characters, and that the blending of these characters in the offspring are largely the result of the fusion of the sexual nuclei. The nuclear fusion is also the basis of all hereditary variation.

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CHAPTER II.—FECUNDATION; MOTILE ISOGAMETES.

ULOTHRIX AND HYDRODICTYON.

There seems to be no question that the simplest and most primitive form of sexuality consists in the union of motile isogametes as found among many of the most primitive algæ. The chief difference between the gametes of such forms as *Pandorina* and *Ulothrix*, for example, and their asexual swarm-spores, from which the gametes were undoubtedly derived phylogenetically, seems to be merely physiological. Generally speaking, the gamete is incapable of developing into a normal adult individual. It must unite first with another gamete of the same species in order to restore the power of growth and division necessary to the development into an individual common to the species, and apart from theoretical considerations (I refer to the number of chromosomes which, of course, has not been determined for these lower forms) this is the most fundamental distinction made. Many other well-known forms among the green algæ might have been taken as representatives, instead of the two selected, but these have been chosen because the development of the reproductive cells from the mother-cell has been more carefully worked out here, and because the processes in this development are coming to be regarded as more important from a genetic standpoint.

In connection with *Ulothrix* I have selected *Hydrodictyon* in order to present the cytological processes preparatory to the formation of gametes in uninucleate as well as in multinucleated cells.

The cytological development, leading to the formation of gametes and also asexual swarm-spores among the simpler representatives of the green algæ, has been investigated by a number of earlier observers, among whom were Alexander Brown, Cohn, Pringsheim, Dodel, Strasburger, Klebs, and lately by Timberlake.

The well-known and widely distributed *Ulothrix* consists of a simple unbranched filament differentiated into base and apex (Fig. 17, A). The cells, except the basal one, which is modified as an organ of attachment, are quite alike. Each contains a single nucleus and a band-shaped chloroplast in the form of an almost complete hollow cylinder. Almost any vegetative cell of the filament save the basal one may, without undergoing any external modification, function as a gametangium.

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The process of cell-formation by which the gametes are developed from the protoplast of the gametangium has been observed and described in some detail by Dodel ('76) and by Strasburger ('92). These authors agree that the gametes arise not by the process of free cell-formation, as understood at the time, but by successive bipartitions of the entire plasmic contents of the cell. According to Strasburger ('92) the process of division in the development of the swarm-spores, which is exactly the same for the gametes, differs from the beginning in a very marked way from the vegetative cell-divisions. At first the cell-contents undergo apparently a sort of rejuvenescence by which the protoplast becomes independent of both the outer and inner plasm

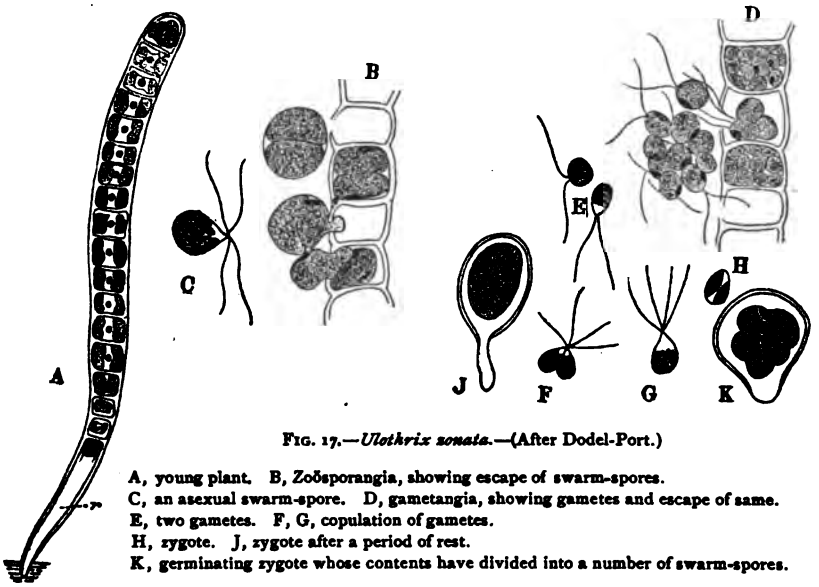

FIG. 17.—*Ulothrix zonata*.—(After Dodel-Port.)

A, young plant. B, Zoösporangia, showing escape of swarm-spores.
C, an asexual swarm-spore. D, gametangia, showing gametes and escape of same.
E, two gametes. F, G, copulation of gametes.
H, zygote. J, zygote after a period of rest.
K, germinating zygote whose contents have divided into a number of swarm-spores.

membranes. In the first division the granular plasma only is halved. The outer plasma membrane (Hautschicht) is undivided, and the membrane surrounding the vacuole remains unchanged. By further successive divisions these two protoplasts give rise ultimately to the gametes. The process of division is the same whether gametes or asexual swarm-spores result (Fig. 17, D). Strasburger has expressed the opinion that, in the development of the gametes, only one more cell-division is necessary above those required for the zoöspores, and this division renders the resulting cells or gametes incapable of further independent development. In what way this last division incapacitates the gametes for further independent development was not discussed at the time. That view was probably prompted by Weismann's

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theory of a reduction division of the chromosomes, which at the time received a wider acceptance than at present.

In *Hydrodictyon* Klebs ('91) affirms that the process of cell-formation, giving rise to gametes or asexual swarm-spores, occupies an intermediate position between simultaneous and successive cell-division. From what follows it will be seen that the process is a cleavage similar to that occurring in certain Phycomycetes, but, using the methods that he did, Klebs failed to perceive the true nature of the process. His account in substance is as follows:

The first indication of cleavage is manifested in the appearance of numerous small clefts, pointed at the ends, in the plasma layer containing the chlorophyll (Fig. 18, A). This can be seen in material cultivated in darkness in a maltose solution, especially after the application of a weak plasmolysing agent. These clefts soon become longer and more numerous, neighboring ones thereby uniting with each other, so that finally the entire chlorophyll-bearing layer is segmented into pieces which are still connected, however, by fine plasmic threads. The cleavage is not confined solely to the chlorophyll-bearing layer, but extends into the colorless plasma in which the nuclei are situated. The plasma membrane and the wall of the vacuole are, on the contrary, unaffected. Previously to and during the cleavage the plasmic layer concerned frequently undergoes a contraction, thus giving rise to colorless spaces, so that this layer appears as a coarse net, as Pringsheim ('71) has described for *Bryopsis*. These spaces contain also some plasma, and, as the plasma membrane and wall of the vacuole are continuous, the entire cell contents form still a unit, as shown by plasmolysis. The continuation of the cleavage results in the segmentation of the plasmic contents into numerous bands with irregular and sinuous contour (Fig. 18, B). These bands undergo still further segmentation (Fig. 18, C), until finally the plasmic contents are broken up into numerous small pieces, each containing a nucleus, which ultimately separate and develop into gametes (Fig. 18, D). The method of division in these portions referred to in Fig. 18, B, C (Klebs continues), appears to consist in a constriction, progressing from one side, but not entirely completed, since the individual parts remain in communication; yet direct observation shows also that, in the plane of division, a colorless line or furrow is frequently present, which gives the impression that the constriction may proceed from within. The same principle operating in the segmentation of the bands or pieces obtains also in the earlier cleavage of the whole plasmic layer of the cell. There is from beginning to end a progressive condensation, but the process that plays the chief rôle is concealed from observation.

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Using improved methods Timberlake ('01) in a study of spore-formation in *Hydrodictyon utriculatum* Roth., has found that, in the earlier stages of the process, cleavage takes place by means of surface constrictions of the plasma membrane on the outside and the vacuolar membrane on the inside of the protoplasmic layer, as may be seen from Klebs' figures (Fig. 18, B, C). The process is a progressive one, the cleavage furrows cutting out first large irregular multinucleated masses of protoplasm, which are in turn divided into smaller ones, until each

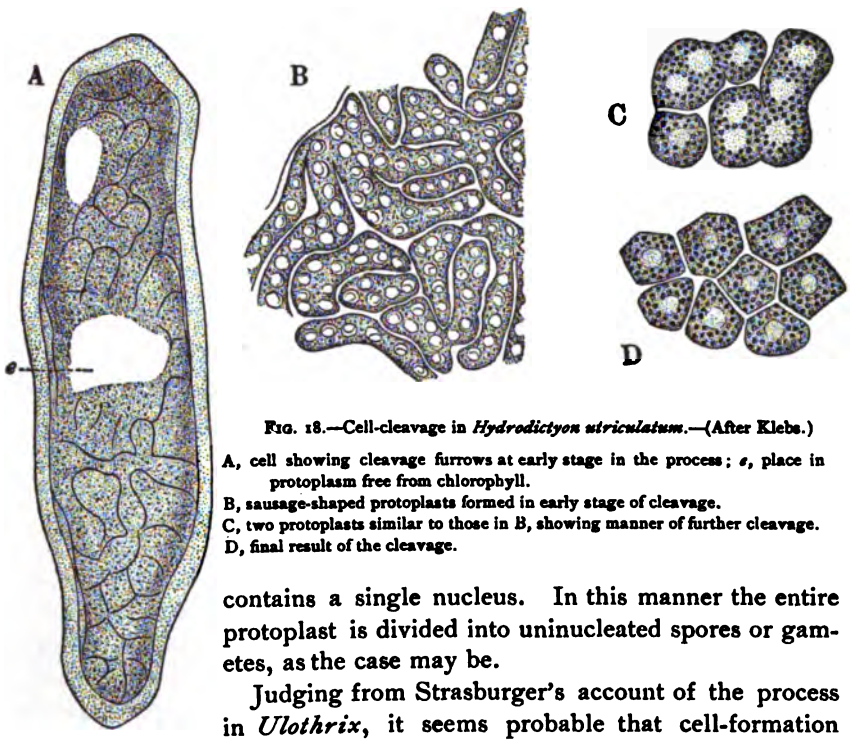

FIG. 18.—Cell-cleavage in *Hydrodictyon utriculatum*.—(After Klebs.)

A, cell showing cleavage furrows at early stage in the process; *e*, place in protoplasm free from chlorophyll.
B, sausage-shaped protoplasts formed in early stage of cleavage.
C, two protoplasts similar to those in B, showing manner of further cleavage.
D, final result of the cleavage.

contains a single nucleus. In this manner the entire protoplast is divided into uninucleated spores or gametes, as the case may be.

Judging from Strasburger's account of the process in *Ulothrix*, it seems probable that cell-formation leading to the development of gametes or swarm-spores is also a cleavage similar to that in *Hydrodictyon*. In *Ulothrix*, however, the cells are uninucleate, and a nuclear division must either accompany or precede cell-division. Until the behavior of the nucleus is known, and the process carefully worked out with the aid of more improved methods, the exact nature of the cell-formation in question must remain largely a matter of conjecture.

In the light of more recent investigations concerning cell-formation among the lower thallophytes, it is evident that our present knowledge of this process in connection with the development of gametes or asexual zoöspores among the algæ is very meager and fragmentary.

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COPULATION OF GAMETES.

The gametes of *Ulothrix zonata* are rounded or oval cells, bearing two cilia at the anterior end (Fig. 17, E). Each contains a nucleus, a red eye-spot, situated about midway between the ends of the cell near the surface, and a chromatophore. According to Strasburger the cilia are developed under the influence of the nucleus and from the anterior, colorless portion or mouth-piece, which consists mostly of kinoplasm. In his later investigation of the subject of swarm cells, Strasburger (1900) finds that the cilia arise from a local kinoplasmic thickening of the plasma membrane at the anterior end. As already mentioned in a preceding paragraph (p. 47), he regards this thickening as the homolog of the blepharoplast of the Archegoniates. In the swarm-spores of *Hydrodictyon*, Timberlake finds a small body at the base of the cilia, which, in some cases at least, was not a part of the plasma membrane.

The gametes copulate in pairs immediately after they escape from the gametangium (Fig. 17, F, G). It is probable that they may be brought together, or at least held together after coming in contact, by means of a chemotactic stimulus. The stigmatic or eye-spots do not unite, but remain separate and independent in the young zygote (Fig. 17, H). There is no doubt of a nuclear fusion, but how soon this takes place after conjugation is not known, so far as the author is aware.

In *Hydrodictyon* the gametes are small, oval in shape, biciliate, containing one nucleus and, according to Klebs, two pulsating vacuoles. They conjugate in pairs immediately on escaping from the gametangium, but I have observed that conjugation may sometimes take place within the mother-cell. If, however, copulation does not follow soon after the gametes are set free, they become incapable of uniting, come to rest and disorganize. Whether this is a rule was not determined.

ECTOCARPUS.

Among the isogamous *Phæophyceæ* the sexual process is doubtless best known in *Ectocarpus siliculosus* Lyngb. from the investigations of Berthold ('81), which have been confirmed and extended by Oltmanns ('99). *Ectocarpus* is of especial interest in this respect, since it represents a transition from isogamy to heterogamy. In fact, there is in the brown algæ, as well as in phylogenetic series of other *Thallophyta*, every transition from the type of gametes found in *Ectocarpus* to that of *Fucus*. The gametes, although nearly or quite the same size and appearing morphologically alike, are physiologically different, and we may, with much propriety, speak of egg-cells and spermatozoids.

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Both Oltmanns and Berthold agree in the opinion that *Ectocarpus siliculosus* may be either monœcious or diœcious, for they observed individuals whose gametes would not conjugate with each other, but only with those of another individual. As is well known, the gametes are generally borne in the so-called plurilocular sporangia. The details in the process of nuclear and cell-division in the development of both gametes and asexual swarm-spores have not, as yet, been thoroughly studied. The gametes (Fig. 19, A) are pear-shaped cells with a chromatophore, nucleus, a reddish brown eye-spot, and two cilia inserted laterally. The cilia are of unequal length, the longer extending forward and the shorter backward.

The conjugation of the gametes can be most readily followed in a hanging drop, into which both male and female gametes are introduced, when the whole process may be observed with the aid of the highest magnifying powers. The female gametes, as a rule, first come to rest, and about each one numerous spermatozoids assemble. If the female gamete comes to rest at the edge of the drop, the male cells cluster about it, attaching themselves apparently by the anterior cilium, giving the familiar picture figured by Berthold (Fig. 19, A). But should the female gamete attach itself to some particle hanging in the arched surface of the drop, this cell then appears as a circular disk surrounded by a wreath of male cells radially disposed. Shortly a male gamete (in exceptional cases two), having attached itself to the female by means of the anterior cilium, approaches the latter apparently by the sudden contraction of the same and unites with it, while the remaining male gametes withdraw (Fig. 19, B, C). In a few minutes cytoplasmic union is complete, and within about ten hours after copulation both nuclei have fused (Fig. 19, E, F, G). The chloroplasts do not unite, a fact which is contrary to the peculiar phenomenon described by Overton for *Spirogyra* (see page 69).

The sexual process in *Ulothrix*, *Hydrodictyon*, and *Ectocarpus* may be considered as fairly typical of the lower algæ in which fecundation consists in the fusion of motile isogametes. In this, probably the simplest and most primitive sexual process, as in the higher plants, it will be seen that fecundation consists in the fusion of the sexual nuclei together with the cytoplasm of the gametes, but the fusion of the nuclei must be regarded as of prime importance.

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CHAPTER III.—FECUNDATION; NON-MOTILE ISOGAMETES.

In this chapter will be discussed the sexual process in several forms in which the gametes are non-motile, *i. e.*, they do not escape from the parent plant and move about in the surrounding media, and are either unisexual or show a certain degree of bisexuality, as in *Basidiobolus*. The forms used, *Spirogyra*, *Cosmarium* and *Closterium* among the desmids, certain diatoms and *Basidiobolus*, have been chosen solely because the development of the gametes and their union have been most thoroughly investigated in certain species of these genera. Owing to the conflicting results obtained by the several investigators in the much-studied *Sporodinia*, the process in this plant, which properly belongs here, will be only incidentally referred to.

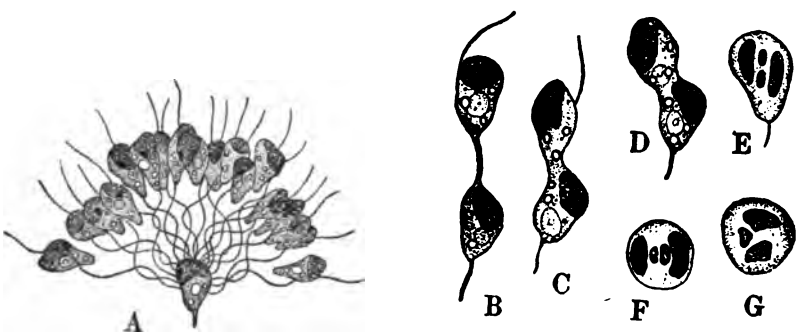

FIG. 19.—Copulation of gametes in *Ectocarpus siliculosus*.
A, female gamete with numerous male gametes attached, seen from the side.
B, C, D, E, successive stages of cytoplasmic fusion.—(After Berthold.)
E, F, G, fusion of nucleus.—(After Oltmanns.)

SPIROGYRA.

Among the algæ *Spirogyra* undoubtedly furnishes the best known illustration of the sexual process in which the gametes are isogamous and non-motile. The process as observed in the living plant has been carefully described long ago by DeBary ('58), Strasburger ('78) and others, and it is now a matter of common observation in almost every botanical laboratory. The nuclear behavior, which cannot be followed in the living specimen, and which is the most essential part of the process, has received comparatively little attention.

Morphologically and physiologically every cell of a *Spirogyra* filament, except those serving as organs of attachment, is exactly like every other cell, so that the filament may be regarded, in a sense at

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least, as a colony of individuals. Any cell of a filament, save those mentioned, may function as a gamete.

In sexual reproduction cells of two filaments lying close side by side send out protuberances toward each other which meet end to end. In the contiguous membranes a circular opening is made by the dissolution of the cellulose walls, through the agency of an enzyme, whereby a continuous canal is formed between the cells (Fig. 20, A). It is highly probable that the conjugating tubes are brought together by the aid of a chemotactic, directive stimulus. Haberlandt ('90) claims, and his view is shared by Klebs ('96), that the conjugating cells exert a mutual chemical influence upon each other, namely, that a cell will put out a conjugating tube only when influenced by another, probably of a different sex, lying near it. In support of this view, Klebs found that cells of individual filaments cultivated upon agar-gelatin, although having been brought side by side by the folding of the filament, never put out conjugating protuberances. A single male filament, on the contrary, may conjugate with several female filaments whenever their cells lie sufficiently near one another, but all those cells of the male filament separated some distance from those of the female remain sterile in spite of the tendency to conjugate. The limits of this mutual action of the filaments (Haberlandt, '90) is equal to a distance of two or three diameters of their cells. Slightly beyond this limit the cells may put out short conjugating tubes, but these never reach each other, the stimulus being presumably too weak. Haberlandt states further that the conjugating tubes are not laid down simultaneously, but rather one sends out a protuberance which calls forth the development of the corresponding tube from the other cell. If the protuberances do not lie exactly opposite, they bend slightly in order to meet each other. A further action of the stimulus is seen when a long male cell copulates with two female cells. Two canals are formed connecting the male with the two female cells, but, of course, only one of the latter receives the gamete. In some species, especially *Spirogyra inflata*, according to Klebs, the meeting of the conjugating protuberances is facilitated by a curving or a knee-like bending of the cells, from whose convex sides the protuberances arise.

These phenomena are not presented in this connection for the purpose of discussing any special phase of the physiology of the sexual process, but merely to indicate a few features manifested by unisexual elements which show a tolerably well-marked tendency toward bisexuality.

When the conjugation canal, joining the gametes, is complete, the turgor in each cell is diminished, so that each protoplast experiences a self-plasmolysis. The contraction usually takes place first in the male

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gamete, which passes through the canal to unite with the stationary or female gamete (Fig. 20, A). Strasburger ('78) has observed that occasionally the female cell was the first to round up. Haberlandt suggests that the extrusion of water is connected with a mutual stimulus between the cells, for the female gamete contracted only when the male was normal, and, furthermore, the male cell became self-plasmolyzed only when connected with a female cell.

The principle underlying the movement of the male gamete through the canal is not well understood. Overton ('88) held that a gelatinous substance was secreted, which, upon swelling, forced the protoplast through the canal. The presence of a mucilaginous substance

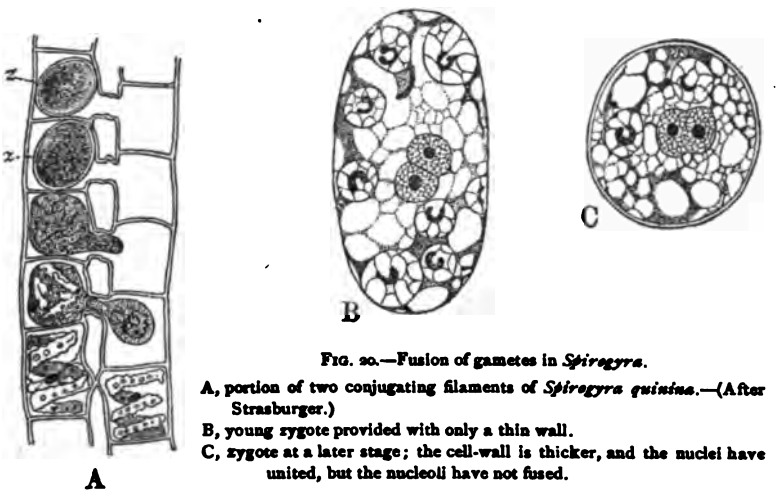

FIG. 20.—Fusion of gametes in *Spirogyra*.

A, portion of two conjugating filaments of *Spirogyra quinina*.—(After Strasburger.)

B, young zygote provided with only a thin wall.

C, zygote at a later stage; the cell-wall is thicker, and the nuclei have united, but the nucleoli have not fused.

has not been demonstrated, however, and it is highly probable that we have to do here with an active plasmic movement operating under the chemotactic stimulus of the two protoplasts. Here fecundation consists in the union of the entire plasma of both gametes, though DeBary records the case of *Spirogyra heeriana*[1] in which a small vesicle of plasma is left beyond the partition wall in the conjugation canal.

Concerning the behavior of the chlorophyll bands in the zygote, much diversity of opinion exists. DeBary ('58) and Schmitz ('82) observed that in species with one chlorophyll band the two chloroplasts united in the zygote to form one continuous band. Overton ('88), on the contrary, asserts that the single band of the female gamete segments at the middle during the fusion of the protoplasts; the two halves then separate, and each piece unites with the ends of the band

[1] See Fig. 10, p. 17, Die Natürlichen Pflanzenfamilien, 1 Theil, 2 Abtheilung.

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furnished by the male gamete. Chmielewskij ('90) finds that in all of the several species examined the chloroplast of the male gamete is dissolved in the zygote, that of the female only remaining.

The behavior of the nuclei during fusion cannot be followed with any degree of certainty in the living specimen. As a rule they cannot be seen at all, a fact which led to the view of the earlier observers that the product of union was without a nucleus. One must, therefore, resort to thin and well-stained sections of properly fixed material to observe the details of nuclear fusion. For this purpose I have selected a small-celled species with one chlorophyll band.

When the young zygote is provided with a thin cell-wall, the two nuclei, which are exactly alike, judging from their appearance, are seen lying closely applied to each other (Fig. 20, B). Each contains a rather large and distinct nucleolus and the characteristic linin net in which are imbedded small granules that behave toward stains as chromatin granules in resting nuclei of higher plants. In fact, the nuclei of *Spirogyra* in this condition seem to possess the same structure as the phanerogamic nucleus. The contiguous parts of the nuclear membranes dissolve or disappear as such, and the network of the one unites directly with that of the other, the fusion of the nucleoli following later (Fig. 20, C). Frequently, before complete union of the nuclei, the wall of the zygospore may become much thickened and less easily penetrated by fixing fluids, so that perfect preparations are difficult to procure. During the development of the zygospore the chloroplasts become vacuolate and the identity of each cannot be made out.

In the preceding paragraphs I have described the nuclear fusion in the zygote as I was able to follow it, but for lack of time and suitable material an exhaustive study of the subject was not made, and consequently I am not prepared to state whether the peculiar behavior of the nuclei as described by Chmielewskij ('92) for *Spirogyra crassa* and *S. elongata* is correct. Chmielewskij states that, as the gametes round up, the nuclear membranes become less distinct, disappearing entirely as the gametes unite. The nuclei now fuse, the fusion being complete by the time the zygote is provided with a thick, dark wall. This fusion takes place during the prophase of division. As soon as fusion is complete the nucleus divides. The daughter nuclei now divide, four nuclei resulting. Two of these then fuse, while the other two divide by direct division and finally disorganize. The fusing nuclei are provided with membranes and are in the resting condition. If the observations of Chmielewskij be true, the process in *Spirogyra* is without parallel in the plant kingdom, at least so far as the author is aware.

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SPORODINIA.

Morphologically considered, the sexual process in *Sporodinia grandis* and in other typical *Zygomycetes* seems to be similar to that in the *Conjugateæ*, but in *Sporodinia* the gametes are multinucleate, and the behavior of the nuclei in the young zygote varies considerably, according to the accounts given by the different observers. After the cytoplasmic fusion of the gametes, the nuclei of each arrange themselves into a spherical layer surrounding a globule of oil, and then fuse, producing a hollow sphere full of oil, which Léger ('95) has called an embryonic sphere (*sphère embryonnaire*). These embryonic spheres lie near the poles of the zygote. During the germination of the zygospore the two embryonic spheres fuse. The fused mass reveals numerous nuclei, which pass into the sporangiferous mycelium and begin to divide. In the azygospore only one embryonic sphere is developed. Wager ('99) regards the union of the nuclei to form the embryonic sphere as the sexual act, and the azygospores are, therefore, truly sexual, the process of conjugation being of secondary importance. Dangeard ('94, '95) does not accept Léger's interpretation of the embryonic spheres, holding that the fate of the nuclei has not been determined.

According to Gruber ('01) no embryonic spheres are to be seen in the newly formed zygote. The numerous nuclei, on the contrary, are uniformly distributed throughout the cytoplasm. After five or six weeks the same condition of things was still found to exist, and what took place finally among the nuclei Gruber was unable to determine. Neither fusion, disorganization nor division of the nuclei was observed even six months after the fusion of the gametes.

From what is now known concerning the sexual union of multinucleate gametes in other groups of plants, in which the sexual process has been unmistakably followed in every detail, it is very probable that a multiple fusion of the nuclei in pairs obtains also in *Sporodinia*.[1]

CLOSTERIUM AND COSMARIUM.

In the desmids the process of fecundation agrees essentially with that described by myself for *Spirogyra*, except as regards the time of the fusion of the sexual nuclei and the behavior of the chromatophores in the zygospore. During the development of a firm cell-wall about the zygote, according to Klebahn ('91), the chromatophores undergo a marked change, the result of which is the formation of two large rounded balls, which are at first rich in starch and of a yellowish color. The part taken by the four original chromatophores in the

[1] See Chapter III, *Albugo Bliti*, and Chapter IV, *Pyronema*.

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formation of these balls was not determined. The union of the nuclei, which are in the resting stage, does not take place until the germination of the zygote. The behavior of the fusion nucleus, although somewhat beyond the province of our subject, is of such a nature as

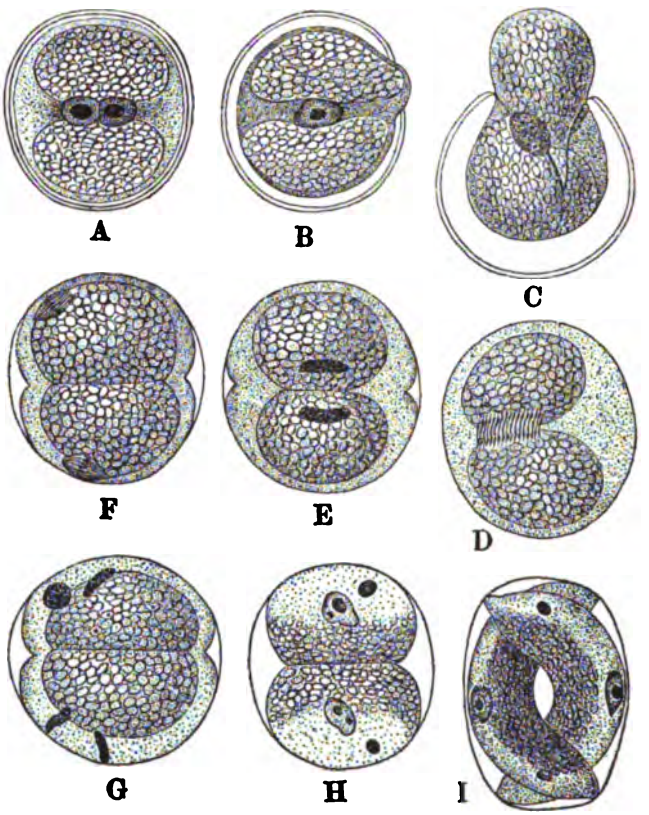

FIG. 21.—Fusion of sexual nuclei during germination of the zygote in *Closterium*.—(After Klebahn.)

A, mature zygote with two large chloroplasts, the two sexual nuclei in contact.
B, beginning of germination; the sexual nuclei have fused while in the resting condition.
C, contents escaping from old wall of zygote; fusion nucleus in prophase of division.
D, protoplast free from wall of zygote, fusion nucleus in anaphase of division.
E, daughter nuclei reconstructed, division of cell begun.
F, spindle stage of second mitosis; the nuclei lie on opposite sides of cell near periphery.
G, second mitosis complete.
H, cell-division has taken place; in each daughter cell one of the two nuclei is much smaller and denser; the two large nuclei are provided with membranes.
I, the daughter cells have begun to assume form of adult cell; in each the large nucleus which persists as the nucleus of the cell, has taken a central position; the smaller one lies near one end of cell.

to merit attention, especially in connection with the nuclear behavior previous to the sexual process in the diatoms to be mentioned below.

The union of the sexual nuclei in *Closterium* and *Cosmarium*,

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according to Klebahn, occurs just prior to the escape of the contents of the zygote from the outer membrane (Fig. 21, A, B). During the latter process the fusion nucleus often shows signs of approaching karyokinesis (Fig. 21, C). There now follow two karyokinetic divisions in rapid succession, so that each daughter cell may contain two nuclei (for a cell-division may also have taken place) one of which remains as the nucleus of the daughter cell, while the other gradually undergoes disorganization (Fig. 21, D, E, F, G, H, I). (See explanation of figure for details.)

It will now be seen that the process in the zygote of the desmids differs from that described for *Spirogyra* by Chmielewskij (see p. 70): (1) in the fusion of the sexual nuclei in the resting stage; (2) in that there is no second fusion of two of the four daughter nuclei, but a cell-division, one nucleus going to each of the daughter cells.

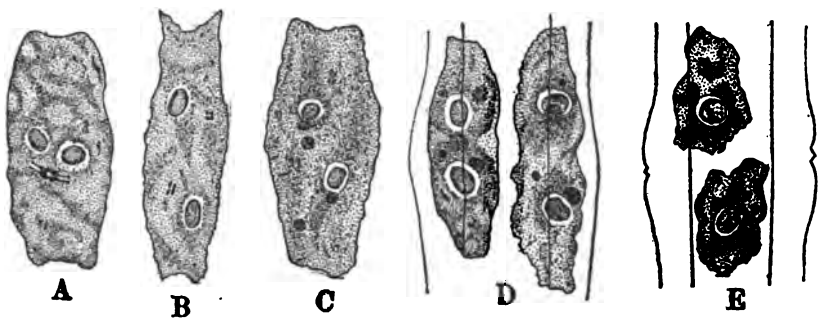

FIG. 22.—Formation of gametes in *Rhopalodia gibba*.—(After Klebahn.)

A, protoplast of cell showing first mitosis; nucleus in spindle stage.
B, second mitosis, each daughter nucleus dividing.
C, second mitosis complete, the four nuclei about equal in size.
D, part of two conjugating individuals; the protoplast of the one on left has begun to divide by becoming constricted in the middle; two nuclei in each cell are large, other two have become smaller.
E, cell-division complete.

DIATOMS.

In the diatoms the type of isogamous fecundation resulting in the formation of the auxospore recalls the nuclear history subsequent to fecundation in the desmids. As in the case of the desmids we are indebted also to the investigations of Klebahn ('96) and to those of Karsten (1900), for a more accurate knowledge of the nuclear behavior preceding the sexual act. The nuclear activity, which immediately precedes conjugation, is of prime importance here, and it is to this that our attention is especially directed.

In *Rhopalodia*, the form studied by Klebahn, two individuals place themselves side by side, being held together by means of mucilaginous masses. The protoplast of each cell, which contains one nucleus and

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in general two pyrenoids, undergoes a rejuvenescence and finally divides. Prior to this cell-division, however, two successive mitotic divisions of the nucleus[1] take place (Fig. 22, A to E). After the first mitosis the daughter nuclei generally move apart toward the ends of the cell whither the pyrenoids also wander (Fig. 22, B). Soon the second mitosis takes place, when four nuclei similar in appearance are present in the protoplast, which may, as yet, show no sign of division (Fig. 22, D). With further progress the protoplast in each individual becomes constricted near the middle and finally divides, two daughter nuclei passing into each daughter cell, which contains one or some-

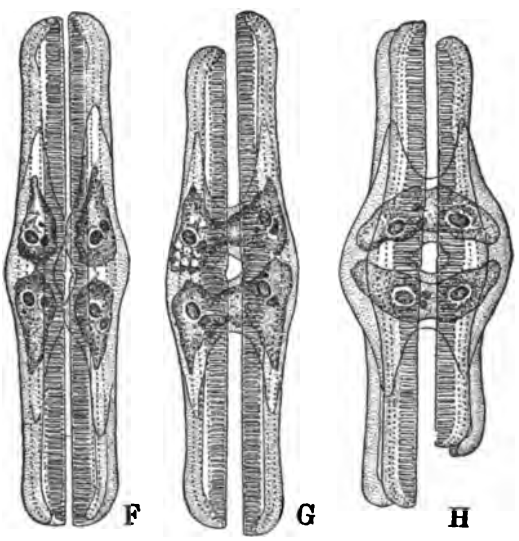

FIG. 23.—Conjugation of gametes in *Rhopalodia gibba*.—(After Klebahn.)

F, conjugating pair seen from valve side; protoplast of each has divided into two daughter cells or gametes; each gamete contains, besides the large pyrenoid, a large and a small nucleus.

G, cytoplasmic fusion of the two pairs of gametes has taken place; the small nuclei are scarcely recognizable.

H, a later stage; the small nuclei have entirely disappeared, while the two functional nuclei in each zygote, which has now changed from a dumbbell to an elongated form, have come nearer together.

times two pyrenoids and a chromatophore (Fig. 22, E). A marked change is now manifested in the nuclei. Of the two nuclei in each daughter cell, one increases in size while the other diminishes, becoming dense and contracted (Fig. 22, D, E). The next step in the process is the conjugation of the daughter cells of one individual with those of the opposite one by means of protuberances sent out from the respective cells (Fig. 23, F, G). The large nucleus of each

[1] For details of mitosis see the original paper of Klebahn, '96.

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cell, followed by the pyrenoid, passes into the isthmus or connecting portion of the dumbbell-shaped zygote, which soon becomes cylindrical or crescent-shaped, and scarcely a trace of the small nuclei are to be seen (Fig. 23, H). During the development of the zygote into an auxospore, the two large functional nuclei assume the structure characteristic of the resting stage (*i. e.*, each presents a granular framework and a definite nucleolus) and fuse. The fusion does not take place in every case at a certain developmental stage of the two auxospores, but may occur earlier in one than in the other (Fig. 24, I, J). As a rule, however, the fusion is complete when the siliceous valves have begun to develop. The behavior of the small nuclei would seem to indicate that they are utilized as food.

A slightly different process, leading to the production of the auxospore, is met with in *Cocconeis placentula* Ehr., as described by Karsten (1900). In this species the protoplasts of the conjugating cells do not divide, and, therefore, only one zygote results. In each cell there is also but one division of the nucleus instead of two as in *Rhopalodia*. Preparatory to the cytoplasmic union the protoplast of each cell contracts. Each cell is seen to possess two nuclei, one large and one small, so that nuclear division must have taken place at an earlier stage. During the contraction mentioned each protoplast surrounds itself with a gelatinous envelope. Near the point of contact of the two individuals the two halves of each shell separate slightly. From the opening in one of the cells, which is regarded as the male gamete, a small papilla protrudes, which grows toward the opening in the female cell, and the gelatinous envelopes are soon in open communication. The entire protoplast of the male cell now passes through this narrow channel into the female cell. The young zygote then increases considerably in size, and begins the formation of a firm cell-wall about itself. Of the four nuclei only the two large ones are now to be seen, the smaller ones having gradually disappeared. The two large functional nuclei, each with a nucleolus, begin to fuse slowly, and, by the time the shell of the zygote is fully formed and the two chromatophores are reduced to one, fusion is complete.

From the foregoing it is clear that the nuclear behavior immediately preceding the sexual act in *Rhopalodia* is strikingly analogous to the process following fecundation in *Closterium* and *Cosmarium*. Whether these processes bear any closer relation to each other than mere analogy is a difficult question. It may be suggested that, in the case of the diatoms, we have to do with the development of two perfect gametes in each cell instead of four, a process similar to that in certain *Fucaceæ*, where only part of the egg-cells in the oögonium mature,

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the others being disorganized; and in the desmids only two out of the four in the germination of the zygote develop into perfect cells.

It is not known whether the reduction in the number of chromosomes, if a reduction actually occurs in either desmids or diatoms, is in any way associated with the nuclear divisions in question, as has been assumed by some authors (see Wilson, "The Cell," p. 198); consequently, in the light of our present knowledge, it cannot be said with any certainty that these nuclear divisions represent a preparation for the sexual act, that in the diatoms taking place just before fecundation while in the desmids it occurs at the beginning of an ontogenetic development.

BASIDIOBOLUS.

A sexual process similar to that in the *Conjugateæ* is found in *Basidiobolus*, one of the *Phycomycetes*. I have selected *Basidio-*

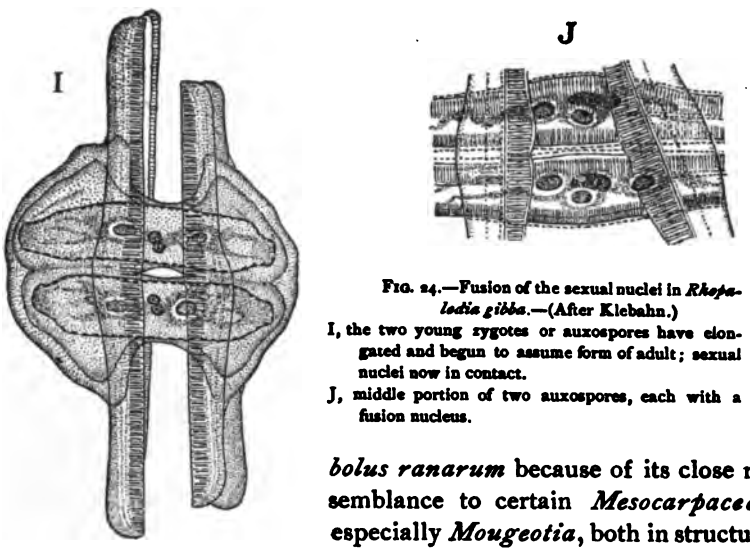

FIG. 24.—Fusion of the sexual nuclei in *Rhopalodia gibba*.—(After Klebahn.)
I, the two young zygotes or auxospores have elongated and begun to assume form of adult; sexual nuclei now in contact.
J, middle portion of two auxospores, each with a fusion nucleus.

bolus ranarum because of its close resemblance to certain *Mesocarpaceæ*, especially *Mougeotia*, both in structure (the cells possess only one nucleus) and in the sexual process, and because the development of the sexual organs and the fusion of the gametes are well known in detail. Sexuality in this genus has recently been subjected to a critical study by Fairchild ('97), whose results form the basis of the following account.

Two neighboring cells of a filament send out near the transverse wall a beak-like protuberance, into which the nuclei of the respective cells pass (Fig. 25, A).

The nucleus in each of the protuberances now undergoes a karyokinetic division, which is followed by the formation of a transverse

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wall cutting off a small cell at the end of the beak (Fig. 25, B). The manner in which this wall is laid down is worthy of special notice here, since it is formed as in the higher plants, namely, through the instrumentality of the kinoplasmic connecting fibers, appearing at first as a cell-plate. Apart from *Chara* this is the only instance as yet known among the lower cryptogams in which a cell-plate is thus formed. Immediately the nuclei have entered the beaks, and prior to the prophase of the nuclear division just mentioned, and also before an increase in size of the female gamete, a hole is formed in the transverse wall separating the two gametes.

The two daughter nuclei cut off in the ends of the beaks gradually disappear, while the other two pass down deeper into the cytoplasm

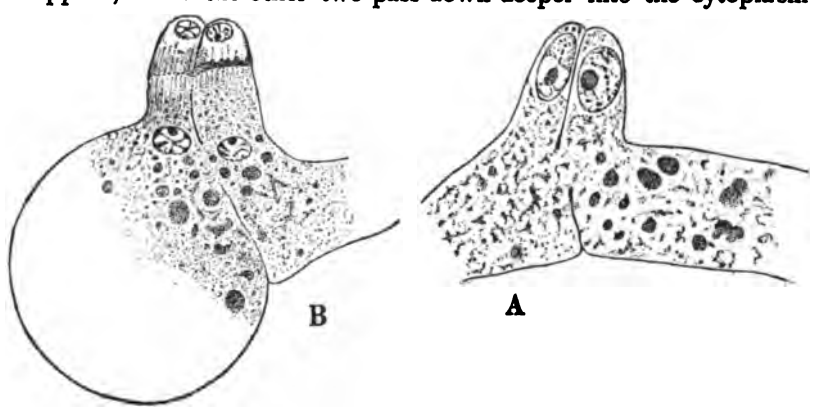

FIG. 25.—Formation of gametes in *Basidiobolus ranarum* Eidam.—(After Fairchild.)

A, two gametes showing the beaks; the nuclei, which are in the beaks, are in the resting condition; the hole has already formed between the gametes.

B, the nuclei have divided and two of the daughter nuclei are cut off in the ends of the beaks, while the other two, which have increased in size, have passed down near the opening in the transverse wall; the female gamete has increased greatly in size, the male retaining its former dimensions.

of the cells (Fig. 25, B). The male nucleus now passes through this opening and comes in contact with the female nucleus (Fig. 26, C). During these movements the nuclei attain their original size, and each contains one or more interwoven nuclear threads, in which chromatin granules are situated at rather long intervals. In this condition the two nuclei remain some time before fusing. The entire cytoplasm of the two gametes is utilized in the formation of the young zygospore, which now forms about itself a very thin wall, within which the thick endospore, consisting of several layers, is gradually developed. Owing to the difficulty with which fixing fluids penetrate the thick wall of the zygote the exact time of fusion of the male and female nuclei is not easily determined, but as the zygospore approaches maturity the fusion is complete, so that no trace of male and female

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nuclei can be distinguished (Fig. 26, D). According to Raciborski ('96) the fusion may be delayed until the germination of the zygote.

The full significance of the formation of the beaks into which the nuclei wander, the division of the latter, and the cutting off of the small cells which degenerate, can be more fully understood only after the process of sexual reproduction is known in other and related forms. The two small cells cut off in the ends of the beaks may, however, be

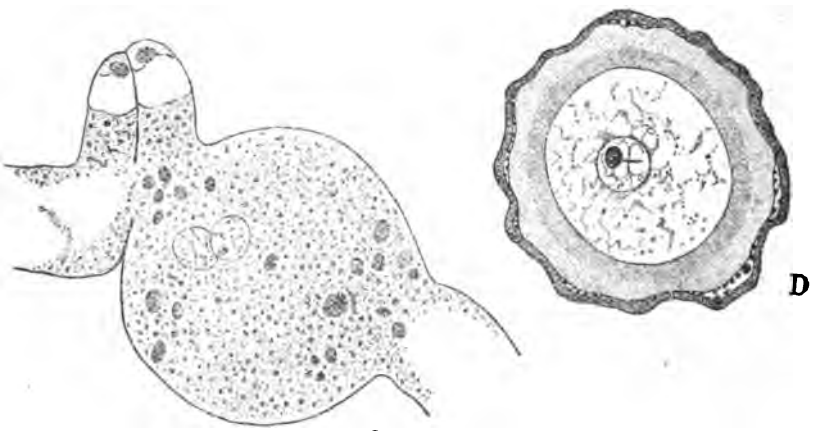

FIG. 26.—Fusion of sexual nuclei in *Basidiobolus ranarum*.—(After Fairchild.)
C, the sexual nuclei are in contact. D, zygote with fusion nucleus and thick cell-wall.

reasonably regarded as degenerate gametes, although it may seem idle to attempt to explain or to bring into line the various peculiar phenomena brought out in the several preceding paragraphs that pertain to the desmids, diatoms, *Basidiobolus* and *Spirogyra*. In the desmids, diatoms and *Basidiobolus*, it is possible that all these phenomena may have resulted independently from similar causes acting during a large part of the phylogenetic history of the respective groups of plants.

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CHAPTER IV.—FECUNDATION; HETEROGAMETES.

In the preceding chapters we have considered sexual reproduction in certain of those *Thallophyta* in which no very marked differentiation of the gametes has been attained, although in *Ectocarpus* especially, and even in *Spirogyra* and *Basidiobolus*, a tendency toward a differentiation into male and female cells is manifested. Nor have we found any modification of the cells bearing the gametes into differentiated sexual organs, unless the gametangia of such forms as *Ectocarpus* be so considered, and even then there is no apparent difference between male and female gametangia. As already mentioned in the introductory chapter, the terms *male* and *female* sexual cells are essentially the expression of a certain fundamental kind of division of labor, and in the developmental history of sexuality in plants we find this division of labor manifested in the gametes themselves before a corresponding differentiation is apparent in the organs bearing them.

SPHÆROPLEA.

Among the algæ one of the best known and most interesting examples of this fact is illustrated in *Sphæroplea annulina*. To Ferdinand Cohn ('55) is due the credit of having established the fact of sexual reproduction in this genus, a phenomenon among the algæ little known at the time. Later *Sphæroplea* was studied by Heinricher ('83), Rauwenhoff ('88), Kny ('84) and more recently by Klebahn ('99). Although both Heinricher and Rauwenhoff followed the behavior of the nucleus during certain stages in the development of the sexual cells and in fecundation, yet in many respects their work was incomplete. For a more thorough investigation of this process, however, we are indebted to the researches of Klebahn, who studied the two varieties of the species, *S. annulina* var. *braunii* (Keutz) Kirchner and *S. annulina* var. *crassisepta* Heinricher. The chief interest in the sexual reproduction of this plant centers upon the fact that in var. *braunii* several nuclei are usually present in the egg-cell.

The contents of the multinucleate cells of *Sphæroplea* present the well-known and characteristic arrangement: In typical cases the central cavity of each cell is traversed by a row of large vacuoles interspersed by smaller ones of varying size. The protoplasm, which forms only a thin layer between the larger vacuoles and the cell-wall, is collected into dense ring-like or band-shaped masses between the

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former. These plasmic rings or diaphragms communicate with each other by plasmic strands or bridges. In the plasmic rings are located the rounded chloroplasts, pyrenoids and the nuclei. Of the latter the number in each ring varies from 3 to 20 in var. *braunii* and from 1 to 4 in var. *crassisepta* (Fig. 27, A).

In those cells in which spermatozoids are developed the nuclei undergo four or five karyokinetic divisions,[1] so that ultimately about 300 small nuclei are present in each band (Fig. 28, A to F). During these divisions the pyrenoids disappear, and the chromatophores undergo several divisions and assume a pale, yellowish-brown color.

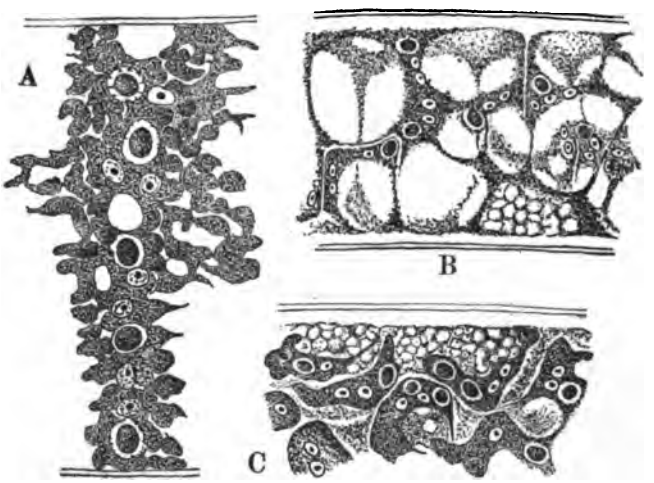

FIG. 27.—Cell-cleavage leading to formation of egg-cells in *Sphæroplea braunii*.—(After Klebahn.)
A, outer view of a protoplasmic ring of a vegetative cell, showing chromatophores, pyrenoids and nuclei.
B, portion of an oögonium showing frothy nature of protoplasm and early stages of cleavage.
C, small portion of oögonium, showing irregular protoplasts resulting from cleavage, which contain several nuclei and pyrenoids.

The plasmic rings up to this time retain their original form. Now the cytoplasm segments into numerous protoplasts, the spermatozoids, in such a manner that each spermatozoid receives only one nucleus (Fig. 29, I, J, K, L). The mature spermatozoids (var. *crassisepta*) are as a rule spindle-shaped, being smaller at the anterior end, which bears the two cilia. Near the middle lies the very small and densely staining nucleus (Fig. 29, L). Kny in his Wandtafel, LXIII, figures four or five yellowish chromatophores in each spermatozoid.

The processes leading to the formation of the egg-cells show a marked difference from those taking place in the antheridium. Even

[1] For details of karyokinesis see Klebahn, '99.

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in the two varieties, as will be shown, the cleavage is not the same. In var. *braunii* the ring-like disposition of the protoplasm disappears, while large vacuoles appear, transforming the entire cell-contents into a foamy structure in which larger and smaller strands and masses alternate (Fig. 27, B). In the dense portions of protoplasm nuclei, as well as chromatophores and pyrenoids, are irregularly disposed. Now a cleavage takes place by which the plasmic contents are segmented into irregular protoplasts of varying sizes (Fig. 27, C). These protoplasts contract (the large vacuoles thereby gradually disappearing) and

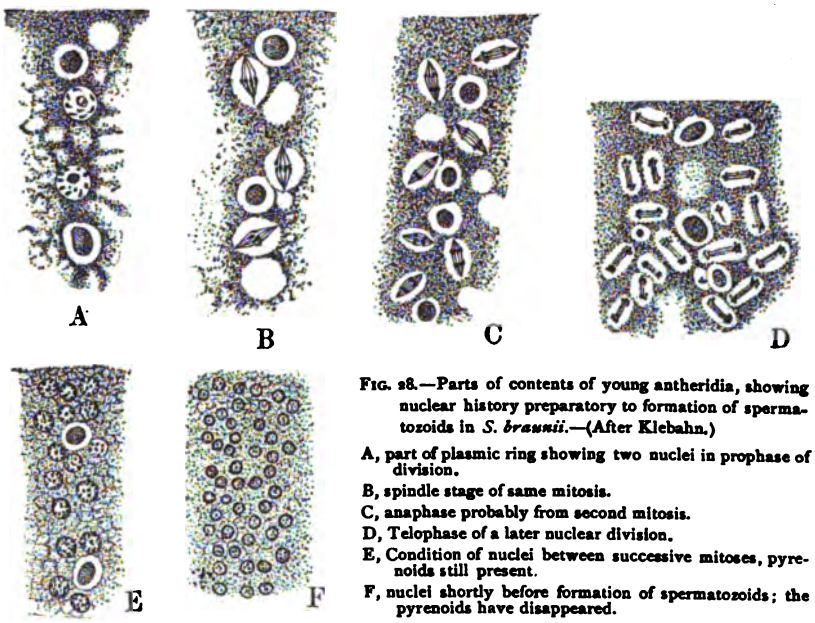

FIG. 28.—Parts of contents of young antheridia, showing nuclear history preparatory to formation of spermatozoids in *S. braunii*.—(After Klebahn.)

A, part of plasmic ring showing two nuclei in prophase of division.

B, spindle stage of same mitosis.

C, anaphase probably from second mitosis.

D, Telophase of a later nuclear division.

E, Condition of nuclei between successive mitoses, pyrenoids still present.

F, nuclei shortly before formation of spermatozoids; the pyrenoids have disappeared.

round up to form the egg-cells, of which two to four are seen in a cross-section of the cell.

Neither shortly before nor during cleavage, according to Klebahn ('99), is there to be observed a division or fusion of the nuclei, so that (contrary to Rauwenhoff who claimed that during the formation of the eggs the number of nuclei was diminished) each egg[1] may contain, in addition to 2 or more pyrenoids, several nuclei, the number varying from 1 to 5 (Fig. 29, A to E). The number of nuclei falling to any egg is largely a matter of chance, since the cleavage planes do not seem to be determined in any way by the number or position of the nuclei in the cytoplasm.

[1] The so-called "giant eggs" are exceptions.

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In var. *crassisepta*, whose cells are smaller (narrower) and with fewer nuclei, the process of cleavage differs somewhat. The eggs in this variety contain, as a rule, only one nucleus. When the protoplasm of the oögonium has become frothy, as described for var. *braunii*, cleavage planes are formed at right angles to the long axis of the cell, thus separating the contents into a row of short segments.[1] Here the cleavage follows in such a way that a nucleus will be included in each segment of the cell, although in exceptional cases two nuclei may be included in a segment. In var. *braunii* we have, therefore, to do with multinucleated eggs, while in var. *crassisepta* each egg-cell is uninucleate.

When the egg-cells are mature, small openings are formed in the wall of the oögonium through which numerous spermatozoids enter (Kny, Wandtafel, LXIV). The manner in which the spermatozoids unite with the cytoplasm of the egg was not observed by the authors cited. According to Klebahn ('99) the fecundated egg is readily distinguished by its delicate membrane and by the presence of the sperm nucleus which appears always in sharp contrast to the nuclei of the egg (these resemble vegetative nuclei) as a small, densely staining body about the size of the nucleolus (*i. e.*, about one micron in diameter) (Fig. 29, A, B). In eggs just fecundated the sperm nucleus lies at the surface beneath the delicate membrane. After a time, the length of which was not determined, the sperm nucleus passes into the interior of the egg, and finally fuses with one of its nuclei (Fig. 29, C, D, E). Before actual fusion the two sexual nuclei remain side by side some time, a phenomenon of very frequent occurrence in the plant kingdom, during which the male nucleus increases in volume, its chromatic substance assuming the form of larger and more distinct granules, until finally the two sexual nuclei can scarcely be distinguished one from the other. The fusion nucleus is easily recognized by its coarsely granular contents, while the other nuclei in the egg appear pale, with a few small granules arranged along the nuclear membrane (Fig. 29, F).

From the foregoing it will be seen that in *Sphæroplea annulina* var. *braunii*, although several nuclei are present in the egg, fecundation consists in the fusion of the spermatozoid nucleus with only one nucleus of the egg-cell. Whether there exists among the several nuclei of the egg any preference in the union with the male nucleus is not known, as there seems to be nothing in the position or appearance of the nuclei which might suggest a preference. The nuclei are irregularly grouped or distributed in the cytoplasm of the egg, and it seems to be purely a matter of chance as to which one will fuse with the sperm nucleus.

[1] See Kny's Wandtafel, LXIV.

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After fusion of the sexual nuclei the oöspore develops its characteristic wall (Fig. 29, G, H). Unfortunately Klebahn was unable to trace the fate of the remaining nuclei. Whether they disappear individually or, after fusion with each other, unite with the fusion nucleus, is a matter of conjecture only. The investigations of Golenken (1900)

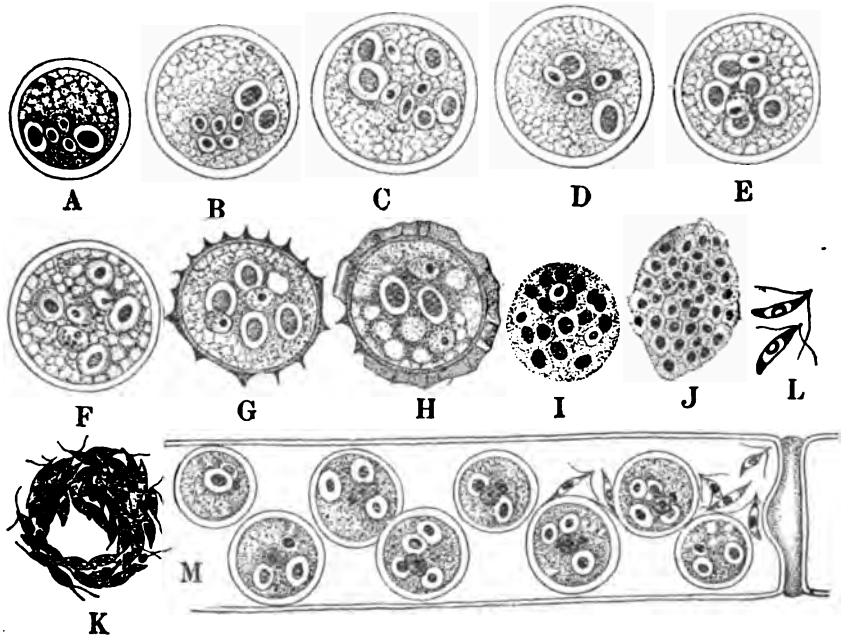

FIG. 29.—Fecundation of eggs and later development of spermatozoids. A–H, *Sphæroplea braunii*. I–M, *S. crassiseptа*.—(After Klebahn.)

A, egg with 3 nuclei, into which a sperm has just penetrated.
B, same stage as A; egg with 5 nuclei.
C, egg with 4 nuclei and 5 pyrenoids; the sperm nucleus has penetrated farther into egg
D, sperm nucleus applied to functional nucleus of egg.
E, fusion of two sexual nuclei.
F–H, maturation of oöspore.
I–K, later stages in development of spermatozoids.
L, two spermatozoids.
M, part of an oögonium showing fecundated eggs and spermatozoids within.

seem to throw further light upon the subject. As reported in the Botanisches Centralblatt, 84, p. 284, 1900, this author, who observed the sexual process in a variety of *Sphæroplea annulina*, which contained multinucleate as well as uninucleate eggs, finds that in the multinucleate eggs the nuclei lie near each other close to the surface, and at a spot where the spermatozoids seem to enter. After fecundation the nuclei first distribute themselves regularly within the egg and then finally fuse to form one nucleus.

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In var. *crassisepta* with uninucleate egg-cells the problem is simpler. The observation of the process in this form in connection with var. *braunii* was fortunate, as it must have served as a control in the interpretation of the phenomena in the multinucleate eggs. If the observations of Klebahn be correct, var. *braunii* represents the only authentic case among the algæ of a normal sexual union of a single male and female nucleus in an egg-cell containing several nuclei of apparently equal morphological value.

FUCACEÆ.

In certain respects the sexual process in *Sphæroplea* is suggestive of that in the *Fucaceæ*. In the latter, however, we have the additional feature that the female gametes or eggs escape into the water, and copulation takes place outside of the oögonium. Probably no other representative of the algæ is so favorable for the observation of the external phenomena of the sexual process than is *Fucus*.

The more obvious details of the process have been observed by Thuret, Oltmanns and others, but it is to the recent researches of Farmer and Williams ('96, '98) that we are indebted for a thorough and comprehensive account of the phenomena to be observed in the living material. The work of these authors supplements also the observations of Strasburger ('97) on the development of the gametes and on the behavior of the sperm-nucleus after it enters the egg.

The type of division of the cell and nucleus in the development of the gametes in this group of plants has been fully treated in the introductory chapter, and the escape of the egg-cells from the oögonium is too well known to bear repetition in this place.[1] Since, however, *Fucus* has figured prominently in recent and much discussed theories bearing upon the significance of the number of the chromosomes in sex and heredity, it is probably not out of place here to state that, in the first nuclear division in the oögonium, the reduced number of chromosomes appears, and that both the nucleus of the egg and the spermatozoid contain this number.

In order to observe the behavior of the sexual cells while alive, and to obtain suitable material for the indirect method of study, Farmer and Williams state:

> Male and female plants were kept in separate dishes, and were covered to prevent drying up. . . . On the appearance of the extruded products, the female receptacles were placed in sea-water, and after the complete liberation of the oospheres a few male branches with ripe antherozoids were first placed

[1] On the method of the liberation of the sexual cells, see Farmer and Williams, '98, p. 629.

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in a capsule of seawater until it became turbid owing to their number. If on examination the antherozoids proved to be active, small quantities were added to the vessel containing the oöspheres. ('96, p. 480.)

When vigorous antherozoids (l. c., '98, p. 631) are transferred to vessels containing healthy oöspheres they at once congregate around them, and attaching themselves to the periphery of the eggs, cause the well-known movements by lashing the water with the free cilium. But, as Thuret noticed, fertilization can often be effected without any whirling movement taking place, and we have observed perfectly normal germination to follow on the addition of apparently inactive antherozoids to the oöspheres.

There seems to be a marked difference between the degree of attraction exerted on the antherozoids by the egg-cells under different conditions. Thus, when the extruded products have been long exposed to a moist atmosphere, so that all the membranes have become deliquescent, the spermatozoids are hardly influenced by the oöspheres. On the other hand the oöspheres which still retain their walls become covered with spermatozoids.

The behavior of the spermatozoids in the genus *Halidrys* is of especial interest in this connection, and I quote again from the same authors (l. c., '98, p. 633):

On watching the behavior of the antherozoids when swimming amongst the oöspheres, they are seen to attach themselves to the surface of the eggs by one cilium, whilst they maintain a circular or gyratory movement around their point of attachment. Most often there is a number—a dozen or more—of these groups, each consisting of 4 to 12 antherozoids, distributed over the surface of each oösphere. The movement is always in the clockwise direction, and the chromatophore is on the end of the antherozoid remote from the egg. The rate of gyration is fairly rapid, 40 to 50 complete turns being made in a minute. After this has been going on for a while the egg itself evinces a change, swelling somewhat and appearing more transparent than before. Sometimes movements of vacuoles may be discerned, and even the position of the nucleus may change. These alterations ensue as the definite result of the stimulus in some way given by the antherozoids themselves. . . . Suddenly the antherozoids are seen to leave the egg like a crowd of startled birds, or else they become quiescent, and these phenomena are immediately followed by a great change in the egg itself, which becomes warty and covered with conical projections. From each papilla a fine thread projects, consisting of a moniliform series of droplets, and the antherozoids may sometimes be observed attached to these threads. After the lapse of a few (3 to 5) minutes the egg resumes its spherical form whilst at the same time its diameter becomes smaller. Still later the fine threads also disappear, whilst the egg regains its original size. As long as the antherozoids are in active motion on the surface of the egg, the latter exhibits a scarcely perceptible rocking movement and is free in the water, but during the events which have just been narrated it

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becomes attached to the surface on which it may be resting. We consider it as certain that the flight of the supernumerary antherozoids marks the moment of actual fertilization, and it seems only possible to interpret the events outside the egg as the results of an excretion from it of some substance which not only exerts on the surrounding antherozoids a negative chemotactic but also a directly injurious effect, for a number of dead sperms may be seen around the fertilized egg. Possibly the bead-like filaments which partly stain like mucilage, are directly concerned in the process.

The facts observed by Farmer and Williams have been given somewhat in detail, because they are suggestive of various interesting problems, especially those pertaining to chemotaxis between sexual cells, a province of physiology well worthy of careful investigation, and one which will undoubtedly yield fruitful results.

It may be noted that, in the attachment of the spermatozoids to the egg by means of one cilium, and in the sudden withdrawal of the supernumerary sperms as if startled, a certain resemblance exists between *Halidrys* and *Ectocarpus* (see p. 66), although these phenomena are less marked in the latter.

In the case of normal healthy products, fecundation occurs within a few minutes after the addition of the male cells. The fecundated eggs form a membrane around themselves at once, and behave in a very different manner from those into which no spermatozoids have penetrated. For example, if the sea-water be gradually drawn off from a mixture of fecundated and non-fecundated eggs, the latter flatten out, their cytoplasm loses its coherence and becomes distributed in all directions, while the former show only local protuberances and burst only at one point.

The passage of the sperm-nucleus through the cytoplasm and its fusion with the nucleus of the egg can be followed with anything like accuracy only in thin and properly stained sections. According to Strasburger ('97), the egg of *Fucus platycarpus* at the time of fecundation is globular and provided with only a plasma membrane. The alveoli of its cytoplasm, together with the included chromatophores, are radially disposed about the centrally placed nucleus (Fig. 30, A), an arrangement which seems to facilitate the movement of the sperm to the egg-nucleus. The passage of the sperm through the cytoplasm and its union with the nucleus of the egg take place rapidly, for both Strasburger and Farmer agree that ten minutes after the addition of the spermatozoids to the water containing the eggs the sexual nuclei have united. Strasburger is inclined to the view that the larger portion of the cytoplasm of the spermatozoid on entering the egg unites

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with its cytoplasm, while the nucleus alone proceeds toward that of the egg. However, the body which approaches the egg-nucleus is wedge-shaped or narrowed slightly at one end. When the sperm-nucleus reaches that of the egg it is about the size of the nucleolus of the latter (Fig. 30, A). It appears as a densely stained and somewhat flattened or lens-shaped body closely applied to the egg-nucleus (Fig. 30, B). An increase in size now follows, during which the denser appearance gives way to that of a less compact structure (Fig. 30, C).

It is now seen (Strasburger, '97, p. 364) that the sperm-nucleus possesses a thread-like framework. With further increase in size the

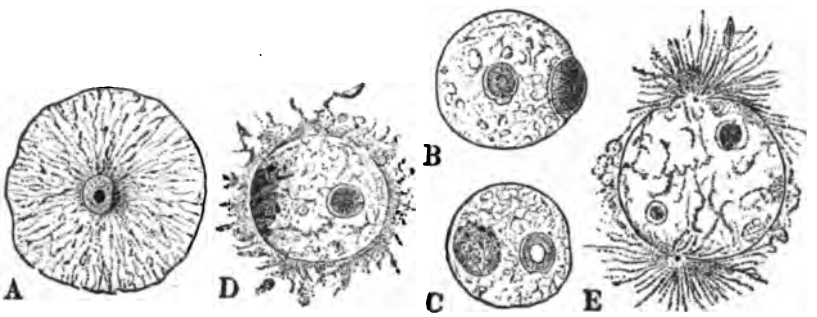

FIG. 30.—Fecundation in *Fucus*. A–D, *Fucus vesiculosus*. E, *F. serratus*.—(After Strasburger.)

A, Egg-cell ten minutes after mixing of sexual elements; male nucleus applied to that of egg.
B, same two nuclei more highly magnified.
C, similar to B; sperm nucleus lies between the observer and egg-nucleus.
D, fusion of nuclei has progressed further; 10 minutes after mixing of sexual elements.
E, 1½ days after fecundation; fusion nucleus preparing for division; poles of future spindle present, but limits of the two nuclei still recognizable; the part derived from male nucleus (on the left) has also a nucleolus.

chromatin thread becomes more prominent, and the boundary between sperm and egg-nucleus gradually disappears (Fig. 30, D, E). In the meantime a nucleolus is found in that portion of the fusion-nucleus coming from the sperm. This is in all probability not brought in as such, but is developed during the process of fusion much in the same way as in the reconstruction of daughter-nuclei following karyokinesis.

In no case observed by the authors mentioned was the sperm-nucleus accompanied by a centrosphere or a system of radiations, either during its passage through the cytoplasm or during fusion. Strasburger ('97, p. 365) states, however, that in some cases he was able to trace the apparent connection between the two centrospheres and the limits of the two sexual nuclei in the oöspore (Fig. 30, E), and he infers that the centrosomes may have been brought into the egg by the sperm in

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an unrecognizable condition. In the light of what is known in certain animal eggs such an inference was tempting, but, from our present knowledge of the centrosphere and centrosome in plants, such a conclusion is no longer justifiable. Moreover, when the centrospheres appear in the first nuclear division of the fecundated egg, it is difficult, and may be impracticable, to distinguish between the male and female portions.

Only in rare cases does more than one spermatozoid enter the egg, for among several thousand preparations examined by Farmer and Williams, only three cases of polyspermy were observed in which two spermatozoids had effected an entrance. The rare occurrence of polyspermy under such conditions as are normal for the plants concerned, and as appears favorable for this phenomenon, would seem to indicate that many cases of polyspermy reported for animals might be largely the result of the prevalence of abnormal conditions at the time of fecundation.

Concerning the large oösphere-like bodies with two nuclei in *Fucus*, which have been regarded by Behrens as fecundation stages, the joint authors cited above state with emphasis that these "represent either abnormally developed oöspheres or oögonia."

VOLVOX.

Without implying any relationship whatever between the two groups of plants to which they belong, the sexual process in *Volvox* may be fittingly mentioned along with that of *Fucus*. In this most highly differentiated representative of the *Volvocaceæ* we have highly specialized sexual cells, and in fact, as has been already stated in a preceding chapter, there is in this group of plants, as in the brown algæ, a gradual transition from the simplest form of sexual reproduction of isogametes to that of the well differentiated bisexual elements of *Volvox*.

Some authors (Strasburger, '92, 1900; Overton, '89) regard the spermatozoid of *Volvox* as a transition between the motile isogametes of algæ and the spermatozoids of the Characeæ. The spermatozoid of *Volvox globator* tapers gradually to a slender anterior end which is colorless, the thicker posterior end being yellowish. At the boundary between the two lies the red eye-spot, and a little farther forward are borne the two laterally inserted cilia. It is reasonable to assume that the cilia spring from a blepharoplast, although positive proof is still wanting. Strasburger (1900, p. 196) regards the colorless and slender anterior end as the homolog of the mouth-piece of algal gametes, from which such highly differentiated bisexual elements as those of *Volvox*

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have been evolved; but in *Volvox* the insertion of the cilia has undergone a lateral displacement, so that they now spring from the base of the mouth-piece.

The large egg-cells, although not escaping from the mother colony into the surrounding water before fecundation, are in a measure free to move passively within the mother colony. The same kind of stimulus operative in bringing the eggs and spermatozoids together in *Fucus* may in all probability obtain also in *Volvox*. In the case of diœcious forms especially, investigation along this line will probably yield important results, and with modern technique a careful study of the behavior of the sexual nuclei and other cytological details of fecundation, concerning which we know practically nothing, will also bring to light much of value and interest to our knowledge of fecundation.

ŒDOGONIUM.

We shall now pass to the consideration of the sexual process in certain of those fresh-water algæ in which the female gamete remains enclosed in its more specialized and characteristic organ, the oögonium.

Beginning with such forms as *Cylindrocapsa* and *Œdogonium* we have a progressive series of forms culminating in *Coleochæte*, in which, apart from the specialized bisexual products, there are more highly differentiated and characteristic sexual organs.

The nature and development of the sexual organs in *Œdogonium* and the process of fecundation have been carefully described by Pringsheim ('56) and others in so far as these phenomena may be followed with accuracy in the living material, but, as regards the more minute structure of the spermatozoid and egg-cell and the behavior of the sexual nuclei in fecundation, the researches of earlier observers leave much to be desired. In more recent years Klebahn ('91) has succeeded in filling in many of the gaps, and it is to his investigations that we are chiefly indebted for a more detailed knowledge of the behavior of the nuclei.

When the oögonium (*Œdogonium boscii*) has attained its definitive form, the protoplasm, which encloses a large vacuole, is everywhere closely applied to the cell-wall. Changes which lead to the formation of the opening in the upper part of the organ are then manifested. Near the spot at which the oögonium will open a small elliptical lamella is formed, which gives a cellulose reaction. The formation of the lamella proceeds from a colorless portion of the cytoplasm, which can not be distinguished at an earlier stage. Between cell-wall and lamella a lens-shaped cavity arises, and a transverse slit

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is formed in the wall (Fig. 31, B). Both cavity and slit are probably the result of a swelling of the wall on the side toward the lamella. The two edges of the slit roll upward and downward respectively, and in this way an opening is formed in the cell-wall. The next stage in development is marked by the contraction and rounding up of the protoplasm to form the egg, but the oögonium is still closed by the lamella. The nucleus lies in the upper end of the egg, and below it is the vacuole, which has become smaller. The nucleus resembles the nuclei of the vegetative cells, being relatively large with a large

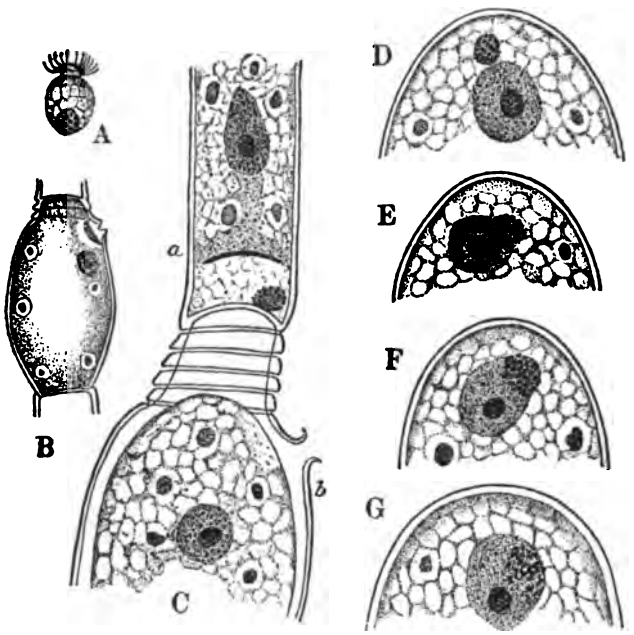

FIG. 31.—Fecundation in *Œdogonium boscii*.—(After Klebahn.)

A, spermatozoid.
B, young oögonium, showing origin of opening in the wall and lamella beneath.
C, oögonium just after opening.
D–G, upper portions of fecundated eggs, showing successive stages in fusion of nuclei.

nucleolus (Fig. 31, C). The so-called receptive spot near the upper end of the egg is formed, according to Klebahn, by the withdrawal of the chloroplasts and not by the collecting of a special mass of cytoplasm. Finally, the closing lamella disappears (probably by being partly dissolved in water), forming an opening for the entrance of the spermatozoids (Fig. 31, C, *b*). No part of the plasmic contents of the egg is expelled on the opening of the oögonium, as has been claimed by some observers. That which is expelled, to judge from Klebahn's

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figure, consists merely of the liquified or gelatinized remains of the lamella.

The spermatozoid, contrary to male gametes among the algæ, bears a circle of cilia at its anterior end (Fig. 31, A). It is not known whether the cilia are developed from a distinct body or blepharoplast, or whether the cilia-bearer is only a thickening of the plasma membrane, as Strasburger maintains for the asexual swarm-spore of this genus. Near the posterior end of the spermatozoid lies its small and dense nucleus, in which a nucleolus is not to be recognized.

Soon after the spermatozoid enters the egg, probably at the receptive spot, its nucleus wanders toward the egg-nucleus (Fig. 31, D, E, F). Before the final fusion of the two nuclei, that of the spermatozoid increases somewhat in size (from 4μ to 6μ) and becomes looser in structure, but a nucleolus was not seen in it. After fusion has taken place, the fact can be readily recognized in that the chromatin elements of the male nucleus are distinguishable in the egg-nucleus. Very soon, however, this characteristic disappears; the male chromatin granules become distributed beyond recognition among those of the egg-nucleus, since both nuclei are in the resting condition.

COLEOCHÆTE.

Coleochæte demands a special consideration not only on account of the peculiarity of the sexual organs but also because this remarkable plant, owing to the behavior of the oösphere subsequent to fecundation, may be regarded as a phylogenetic guide-post, which enables us to connect with each other different groups of thallophytes, and which indicates the probable course traversed by the ancestors of the lower archegoniates.

The recent studies of Jost ('95) and especially those of Oltmanns ('98) have confirmed the classical account of Pringsheim ('58, '60) with the addition of clearing up certain obscure cytological details, which was possible only with the aid of more improved technique.

In the development of the antheridium a small protuberance is formed from the end cell of a filament, into which passes a daughter-nucleus resulting from the division of the nucleus of the mother-cell, and which is cut off by a wall formed at the junction of the protuberance and the mother-cell (Fig. 32, A). No part of the chloroplast of the mother-cell passes into the antheridium. In addition to this central antheridium, others will be formed from the mother-cell in like manner, so that finally several antheridia stand side by side at the end of the mother-cell as so many branches (Fig. 32, B). The spermatozoids,

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of which only one is borne in each antheridium, are, according to Pringsheim ('58, p. 297), almost entirely colorless, with but a faint greenish hue; each bears at the anterior end two cilia, one extending backward during the progressive motion of the cell. In the absence of a chromatophore the spermatozoid of *Coleochæte* differs from that of *Œdogonium*, in which the chlorophyll undergoes a transformation in the male gametes, and in this respect it foreshadows the development of the sperm in higher plants.

The oögonium is also developed from the end cell of a branch. It is recognized first by the presence of a beak at the distal end of the cell, which soon becomes the neck of the flask-shaped organ (Fig. 32, C, D). In the neck dense colorless cytoplasm accumulates which contains one or more large vacuoles. In the basal or ventral portion are situated the nucleus, a large vacuole, and a laterally placed chloroplast. The neck now increases in length with an apparent increase in the quantity of its cytoplasm, the ventral portion remaining unchanged. As soon, however, as the neck has reached its definitive size, a transformation takes place in the ventral part of the oögonium; the chloroplast leaves its lateral position, passes down and applies itself closely to the bottom of the organ (Fig. 33, E). It has increased appreciably in size and contains two pyrenoids. The oögonium opens probably by the gelatinization of the end wall of the neck. As soon as the organ opens the cytoplasm contracts into the basal portion to form the egg-cell. Whether a part of the cytoplasm in the neck is thrown off cannot be stated positively, but there is no reason to believe that this occurs. Both Jost and Oltmanns accord in the opinion that no cytoplasm is expelled when the oögonium opens, while Pringsheim speaks of the extrusion of a colorless substance only, which disorganizes at once. The expulsion of a small quantity of mucilaginous substance, or even cytoplasm, is utterly without important significance, as the nucleus of the oögonium does not divide previously to fecunda-

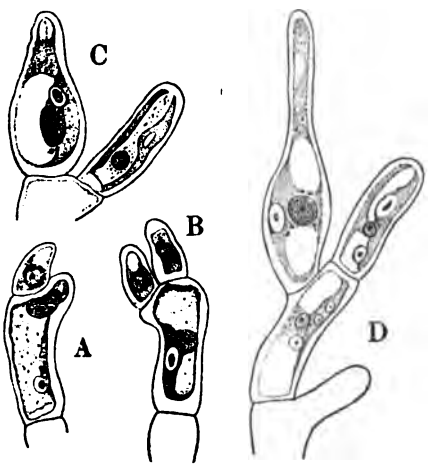

FIG. 32.—Development of sexual organs in *Coleochæte pulvinata*.—(After Oltmanns.)
A, B, development of antheridium.
C, D, two young stages of the oögonium.

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tion. In the withdrawal of the chloroplast into the base of the egg-cell, and the formation of a receptive spot, *Coleochæte* is paralleled by both *Œdogonium* and *Vaucheria*.

Soon after entering the oögonium the spermatozoid penetrates the egg, a membrane is formed about the latter, and the sperm-nucleus wanders toward that of the egg (Fig. 33, F). Before final fusion takes place, one or more changes occur in the egg, which may be worth noting. The chloroplast which lay at the bottom of the egg,

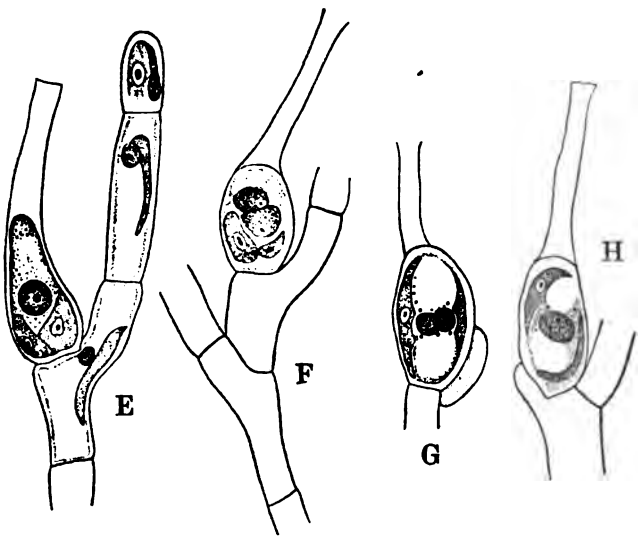

FIG. 33.—Fecundation in *Coleochæte pulvinata*.—(After Oltmanns.)

E, mature oögonium, egg rounded off.
F-H, oögonia with fecundated eggs; male nucleus in F applied to that of egg; both nuclei in resting stage.
G, a little later than F; the chloroplast has taken a lateral position in egg.
H, fusion of sexual nuclei complete.

as previously stated, divides, and the two resulting chloroplasts take positions on opposite sides of the egg (Fig. 33, G). The egg and, consequently, the ventral part of the oögonium increase in size; in the former vacuoles appear, and the nuclei which are in the resting condition fuse completely (Fig. 33, H).

For the further behavior of the oöspore and its germination, which, as is well known, bears a tolerably close resemblance to such liverworts as *Riccia*, the reader is referred to the original papers of Pringsheim and Oltmanns.

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VAUCHERIA.

With the possible exception of *Sphæroplea annulina* var. *braunii*, we have dealt thus far with heterogamous fecundation in those algæ with uninucleate cells. We shall now examine the sexual process in three notable types, one from among the algæ and two from the fungi, namely, *Vaucheria*, *Albugo* (*Cystopus*), and *Achlya*, in which the cells are multinucleate.

In the species under consideration, *Vaucheria clavata*, both antheridia and oögonia may be considered as short side branches cut off from the parent filament by transverse septa. The primordium of the antheridium (Oltmann's, '95) contains numerous small nuclei which probably multiply by division. After the formation of the transverse wall, the nuclei become spindle-shaped, move into the central vacuole, and assume a radial arrangement. Each spindle-shaped body surrounded by a court of fine cytoplasm free from chlorophyll represents a spermatozoid. Very fine threads visible in the antheridium were regarded as cilia.

Concerning the rôle of the nuclei during the development of the oögonium, the several authors differ somewhat. According to Schmitz ('79) the numerous nuclei present in the young oögonium probably fuse later into one. Similar results were obtained by Behrens ('90). Schmitz ('83) claimed that, in the plasmic mass extruded on the opening of the oögonium, small nuclear fragments were present, which had probably become separated from the nuclei of the young oögonium. Klebahn ('92) disputed the above conclusions and asserted that, long after fecundation, he had observed numerous nuclei in each oöspore. Oltmanns ('95), using more exact methods, found that a union of the several nuclei in the young oögonium does not take place, but, on the contrary, all save one pass back into the parent filament before the formation of the transverse wall cutting off the oögonium.

The development of the oögonium, according to Oltmanns, is as follows: Together with the protoplasmic mass numerous nuclei pass into the primordium of the oögonium (Oögonanlage) (Fig. 34, A). The nuclei, which are in the neighborhood of the future beak, probably undergo division, thereby increasing their number. As soon as the oögonium has reached its definite size, a retreating movement of the plasmic mass sets in, and a portion of the plasma, with numerous chloroplasts and nuclei, re-enters the mother-filament (Fig. 34, B). The single nucleus remaining tarries awhile in the beak at the boundary between the colorless and chlorophyll-bearing plasma, but finally

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it wanders toward the center of the oögonium (Fig. 34, C), which is now separated from the filament by a cross-wall. The egg-nucleus retains this position until fecundation (Fig. 34, D); it does not divide and the probability of any nuclear substance being thrown off with the extrusion of a small plasmic or mucilaginous mass when the oögonium opens is, therefore, excluded. Although Oltmanns observed in the cytoplasm of the beak granules staining somewhat more intensely than

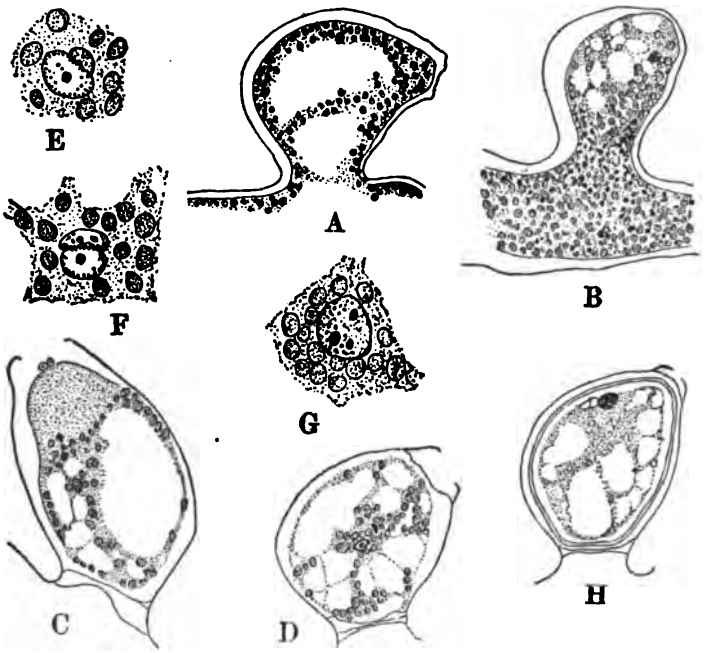

FIG. 34.—Fecundation in *Vaucheria clavata*.—(After Oltmanns.)

A, B, young oögonia before being delimited by transverse walls from filament. In B all nuclei save one are passing back into filament.
C, oögonium ready for fecundation.
D, the spermatozoid has entered egg.
E, F, sexual nuclei in contact; in F the male nucleus has increased in size.
G, a fusion nucleus.
H, oögonium containing oöspore several weeks old.

the rest, yet he does not think it probable that these sustain any relation to the nuclei. At the upper end of the egg is the rather large receptive spot formed by the withdrawal of the chloroplasts from that region.

Immediately on entering the cytoplasm of the egg the sperm-nucleus increases noticeably in size; its linin net, now more loosely arranged, reveals many strongly-staining granules which are probably chromatin. In the meantime the egg-nucleus enlarges considerably, and appears

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more distinctly granular. It contains also a rather large and distinct nucleolus. When the two nuclei come in contact, the male is smaller than the female (Fig. 34, E). Fusion now takes place (Fig. 34, F, G), and the fusion-nucleus presents at first a fine hollow framework in which lie numerous chromatin granules of about equal size; later it becomes smaller and denser, appearing more finely granular, when finally a large nucleolar body is again present (Fig. 34, H).

ALBUGO (CYSTOPUS).

The nuclear behavior and certain cytoplasmic phenomena manifested in the development of the sexual organs, especially the oögonium, of the genus *Albugo* is, so far as known, unique among the Thallophyta, if not in the plant kingdom. The union of several male with several female nuclei in the oösphere of *A. bliti* and *A. portulaceæ* (Stevens, '99, '01) is paralleled among plants only by *Pyronema* (see p. 111) and the possible case of *Sporodinia grandis*. We shall confine ourselves first to the development of the sexual organs and fecundation in *Albugo candida*, referring in a later paragraph to the phenomena described for *A. bliti*, *A. portulaceæ* and other closely related representatives of the group.

The following statements are based largely upon the researches of Wager ('96), probably the most complete account published for this species. The observations of Wager have been confirmed by the later studies of Berlese ('98), Davis (1900) and Stevens ('01), those of Davis and Stevens presenting more clearly certain details regarding the central body of differentiated cytoplasm in the oögonium. The more obvious details in the development of the sexual organs are too well known to bear repetition, and consequently the reader's knowledge of that part of the process is assumed.

The antheridium, which appears almost simultaneously with the oögonium, is more or less densely filled with granular cytoplasm in which several nuclei are present when the partition wall is formed delimiting the antheridium from the parent hypha. Previously to or during the early development of the conjugation-tube, the nuclei undergo a karyokinetic division by which their number is doubled (Fig. 35, A).

When a quantity of cytoplasm and numerous nuclei have passed into the enlarging primordium of the oögonium, a transverse wall is formed separating it from the parent hypha. The cytoplasm shows a foam structure, and the nuclei are more or less regularly spaced in its reticulum (Fig. 35, B). The nuclei possess a membrane, and in

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structure seem not unlike those of higher plants. The number of nuclei in the young oögonium, at this stage, varies with its size, the average being from 70 to 110. The antheridium, containing from 6

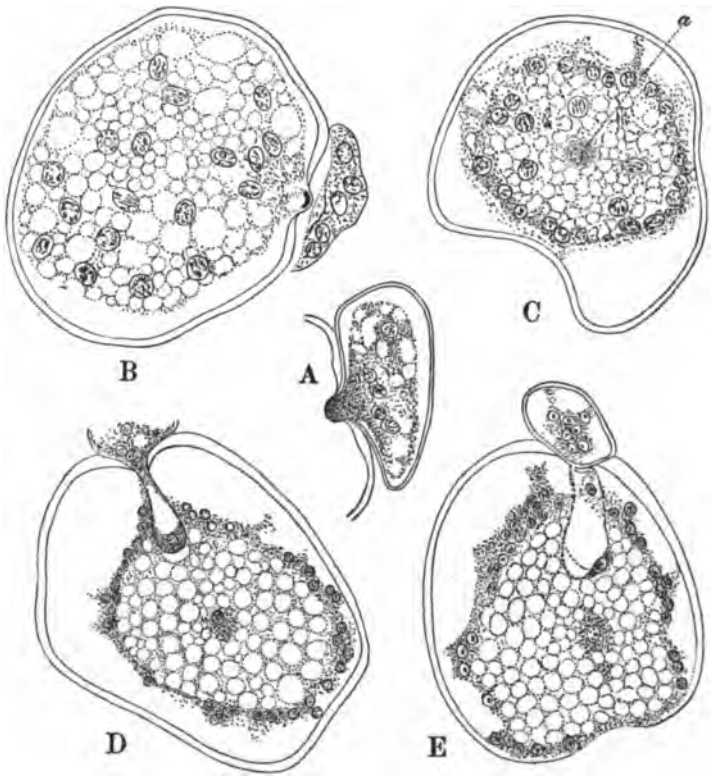

FIG. 35.—Development of sexual organs and fecundation in *Albugo* (Cystopus) *candida*.—(After Wager.)

A, antheridium attached to wall of oögonium, just beginning to push out its conjugating tube; dense mass of cytoplasm and several nuclei seen near projection.

B, young oögonium after its delimination from mycelium, with antheridium attached; receptive papilla projects from oögonium toward antheridium; nuclei seem to be entering prophase of division.

C, later stage; protoplasm has contracted into a large central mass; nearly all the nuclei have divided, and are collecting at periphery of central mass; the deeply stained mass of cytoplasm, *a* (central body, cœnocentrum), is seen in center in contact with egg-nucleus; egg-nucleus is derived from one of the original nuclei of oögonium.

D, oögonium into which conjugating tube has penetrated; differentiation of periplasm and oöplasm becoming apparent, though a plasma membrane has not been formed around the egg; in center of oöplasm is the egg-nucleus near the dense mass of cytoplasm; in end of conjugating-tube is dense cytoplasm in which lies the male nucleus.

E, later stage than D; apical wall of conjugating tube, becoming very thin; plasma membrane of egg not yet formed.

to 12 nuclei, now applies itself to the oögonium. The structure both of its nuclei and cytoplasm is similar to that of the oögonium. Soon after the two organs come into contact with each other, a portion

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of the cytoplasm just beneath the wall of the oögonium on the side nearest the antheridium presents a granular and more homogeneous appearance. At this place a papilla with a deeply stained apical spot is formed, which tends to bore its way through the wall of the oögonium, causing the wall to become thinner. This is called the receptive papilla, since it marks the spot at which the conjugation-tube penetrates the oögonium. It doubtless facilitates the development of the conjugation-tube.

In *A. portulaceæ* (Stevens, '99) this receptive papilla seems to penetrate the antheridium.

The differentiation of the oöspore, which now begins, is manifested in the contraction of the protoplasm toward the center into a rounded mass connected with the wall of the oögonium by thick plasmic strands. This mass contains all the nuclei (Fig. 35, C). It gradually becomes further differentiated into a central vacuolate and reticulate mass, the oöplasm, which becomes the egg-cell or oösphere, and an exterior layer of very dense non-vacuolate cytoplasm, the periplasm. With the exception of a few plasmic strands, which extend to the wall of the oögonium, the entire protoplasmic contents outside the oösphere become finally condensed into periplasm. The nuclei, located mostly in the periplasm and gradually becoming more and more restricted to this layer, now undergo karyokinetic division whereby their number is doubled. Stevens claims that two mitoses occur in both sexual organs during their development.

While nuclear division is taking place a dense granular and rather sharply defined mass of cytoplasm appears in the center of the not yet completely differentiated oösphere (Fig. 35, C, *a*). Wager, '96, says:

> It is of the same nature as the dense protoplasmic mass which appears in the fertilizing tube at the moment when it begins to grow, and is produced probably by an accumulation of stainable granules from the protoplasm. This dense mass of protoplasm can be observed in oögonia of all stages, such as are figured in (l. c.) Figs. 8 and 22. Shortly after its appearance one of the nuclei produced by the division in the oögonium comes into close contact with it, and gradually becomes more or less completely embedded in it. All the other nuclei pass to the periplasm, leaving this single nucleus in the center as the nucleus of the ovum (Fig. 35, D, E).

At this stage the oösphere may be considered as differentiated, although its limiting plasma membrane has not yet appeared.

It seems that this central cytoplasmic body or mass which has received much attention at the hands of later observers was described by Dangeard as an oil globule, and mistaken by Chmielewskij for a

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nucleus. Swingle ('98) called attention to this body in *A. candida*, which he was inclined to regard as an organ of the oögonium, taking some part in the delimination of the egg and the fusion of the male and female nuclei. A similar body has been observed in *A. bliti*, *A. tragopogonis*, and *A. portulaceæ*, by Stevens ('99), who proposed for it the name "cœnocentrum." In *A. bliti*, in which it was described as structureless and unchanging, this body does not seem to be so intimately associated with the sexual nuclei as in *A. candida*, as noted by Wager and Davis. In *A. tragopogonis* it occupies an intermediate position in size between that in *A. bliti* and *A. candida*, where it is largest. According to Davis's figures the female nucleus does not become embedded in the body in question. In *A. candida* this body disappears during the union of the sexual nuclei or a little later.

There is no doubt that these observers refer to the same phenomenon, which is the expression of a specialized and tolerably well differentiated portion of the cytoplasm of the oögonium. It may have to do in some way with the delimination of the egg-cell and, possibly, with the union of the sexual nuclei, but it certainly can not be regarded as an organ of the cell or of the oögonium with morphological rank. Stevens ('01) regards this body as nutritive in character and exerting a chemotactic stimulus upon the sexual nuclei.

During the changes just described the nuclei of the antheridium have been undergoing division, and their number is now about twice that at the beginning. The conjugation-tube has grown and pushed its way through the periplasm into the plasma of the egg. A single nucleus and a small quantity of densely staining cytoplasm pass from the antheridium into the conjugation-tube to its apex (Fig. 35, D). The tube now grows toward the center of the oösphere, around which a plasma membrane has not yet been formed (Fig. 35, E). The dense mass of cytoplasm in the end of the tube becomes reduced in amount, having been used up probably to form the new growing wall (Wager, '96, p. 330). The growth of the conjugation-tube continues until it comes into contact with the central mass of dense cytoplasm (cœnocentrum) referred to in the preceding paragraphs. As soon as the end of the tube comes into contact with the nucleus of the egg the male nucleus is expelled and the tube immediately contracts, or rather collapses, and is withdrawn from or absorbed by the oösphere, leaving a large vacuole to mark its position (Fig. 36, F, *a*). The two nuclei are thus left in close contact with each other, the male being slightly smaller than the female (Fig. 36, F). A delicate membrane, the plasma membrane, now becomes visible around the oösphere, separating it from the dense surrounding cytoplasm, the periplasm. From Davis's

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Fig. 5 (l. c., 1900) it seems that the plasma membrane might be formed at an earlier stage. The sexual nuclei remain close, side by side, for a short time, and then fuse to form the nucleus of the oöspore or fecundated egg (Fig. 36, G).

It will thus be seen that while the antheridium of *Albugo candida* contains several nuclei, only one, together with a small portion of cytoplasm, passes into the egg. The egg, although differentiated within a multinucleate organ, contains but one nucleus, and fecundation consists essentially of the union of one male with one female nucleus.

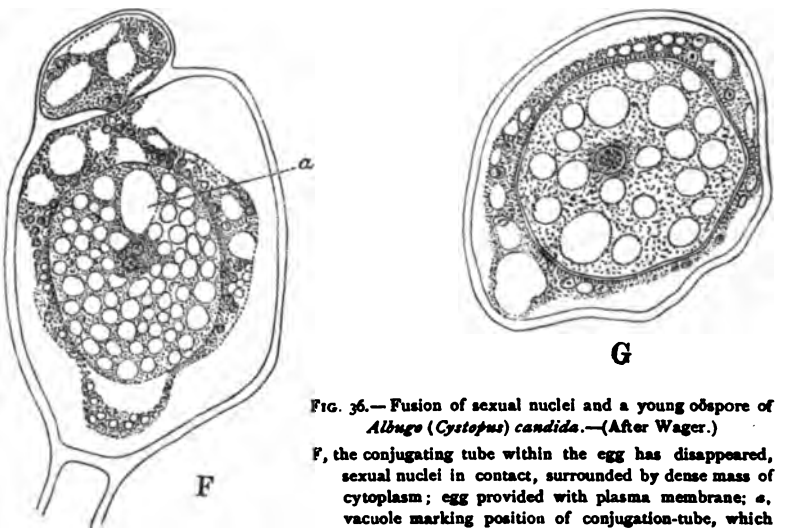

FIG. 36.— Fusion of sexual nuclei and a young oöspore of *Albugo* (*Cystopus*) *candida*.—(After Wager.)

F, the conjugating tube within the egg has disappeared, sexual nuclei in contact, surrounded by dense mass of cytoplasm; egg provided with plasma membrane; *a*, vacuole marking position of conjugation-tube, which has disappeared.

G, young oöspore with fusion nucleus which seems to be in prophase of division.

As already mentioned in a preceding paragraph, a remarkable contrast is described by Stevens as taking place in two other species of *Albugo*, namely, *A. bliti* and *A. portulaceæ*. In the last two species named the differentiated egg-cell is multinucleate, and, since several nuclei enter from the antheridium, fecundation consists in the union of several male with several female nuclei in the same egg. This is the more remarkable, because in all other species of this genus, so far as the author is aware, and in other closely related genera of the *Peronosporeæ*, fecundation consists in the union of one nucleus of each sex. In *A. tragopogonis*, whose mature egg is uninucleate, Stevens finds that the oögonium develops in the same manner as in *A. bliti* and *A. portulaceæ*, but it is reduced to a uninucleate condition by the disorganization of the supernumerary nuclei.

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As stated in the foregoing, the process described for *A. bliti* and *A. portulaceæ* is paralleled in *Pyronema*, one of the *Ascomycetes*. A discussion of the process in this genus will form a part of the next chapter.

Fecundation in the genera *Peronospora* (Wager, 1900) and *Pythium* (Miyake, '01; Trow, '01) bears a close resemblance to that in *Albugo*. In the several species investigated, a receptive papilla is formed by the oögonium during its development. This papilla certainly facilitates in some way the development of the conjugation-tube, which, as all the observers state, is formed by the antheridium. In *Araiospora pulchra*[1] Thaxter, one of the *Leptomitaceæ*, in which the periplasm is developed as a peripheral layer of cells surrounding the egg, there is some evidence which suggests that possibly the conjugation-tube is formed by the oögonium. Wager's Fig. 4 for *Peronospora* seems to lend support to this view as applied to that genus.

A central body of differentiated cytoplasm is present in some degree in all genera, being more prominent, perhaps, in *Albugo candida* and *Peronospora parasitica*. Wager and Stevens have suggested that it is functional in bringing the sexual nuclei together, but when it is known that in *Peronospora parasitica* these nuclei separate again some distance from each other before fusion, it is difficult to understand the necessity of such a body unless it is assumed that stronger forces are at work in the periplasm which tend to bring all nuclei into that region and retain them there, the central body exerting, of course, a stronger chemotactic stimulus upon some particular nucleus which becomes the egg-nucleus, or, in case of several egg-nuclei, as in *A. bliti* and *A. portulaceæ*, upon several particular nuclei. During the development of the sexual organs in the several species in question a mitotic division of the nuclei takes place. In *Pythium ultimum* (Trow, '01) the nuclear division in the antheridium may follow a little later than in the oögonium, thus giving the impression that a second mitosis occurred. The division in both organs seems to be simultaneous in *Pythium de baryanum* and *Peronospora parasitica*. Both Wager and Stevens have expressed the opinion that the reduction in the number of the chromosomes occurs in the antheridia and oögonia, but no decisive evidence is at hand.

In *Albugo candida* the sexual nuclei fuse immediately after the entry of the male nucleus into the oösphere, and the same is true for *Albugo portulaceæ*, *Peronospora ficaria*, *P. alsinearum*, and *P. effusa*, according to Berlese. In *Pythium ultimum*, *P. de baryanum*,

[1] From an investigation made in the botanical laboratory of Indiana University, by Dr. C. A. King.

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and *Peronospora parasitica*, fusion is retarded, taking place only after the egg has developed a tolerably thick wall about itself. The retarded fusion of the nuclei has already been pointed out for *Spirogyra*, *Cosmarium*, *Closterium*, and *Basidiobolus*, and, as will be seen, it is of frequent occurrence in the plant kingdom.

ACHLYA AND SAPROLEGNIA.

The sexuality of the *Saprolegniaceæ* is, perhaps, one of the oldest questions in botany still in dispute. The fact that apogamy obtains in so many species has led observers to accept with the greatest reserve any affirmation of sexuality, although based upon observations which, in other groups of plants, would not be questioned as positive proof of a sexual process.

Pringsheim ('57) was probably the first to attribute to any representative of this group a sexual reproduction, basing his conclusions chiefly upon a study of *Saprolegnia monoica*. He described the development of the sexual organs, the penetration of the oögonium by the conjugation-tubes, and their growth inward among the egg-cells. He stated also that the tubes opened and discharged their contents among the eggs. Reasoning from the analogy of *Vaucheria*, Pringsheim concluded that a real sexual process existed in the species in question.

Several years later De Bary ('81) combated this view, alleging that, as he did not observe the fusion of the conjugation-tubes with the egg-cells (*Saprolegnia ferax* and *Achlya polyandra*), no fecundation took place and that apogamy characterized the entire group. De Bary made a careful study of several species, keeping pure cultures of the same running for several years, and his view, it is safe to say, has been more generally accepted by botanists than that of Pringsheim.

Pringsheim continued his studies, and in 1882 brought forth additional evidence in support of his view. He described and figured the fusion of the conjugation-tubes with the egg-cells in *Achlya polyandra*, and, although his "spermamœba" were nearly amœboid parasites and not male gametes, as he persistently maintained, yet his collected observations seemed to furnish as strong evidence in favor of sexuality as that which could be brought against it by his opponents. Since the above mentioned publications of Pringsheim and De Bary the majority of observers dealing with the subject have leaned toward the view of De Bary.

Within more recent years the subject has been taken up by Hartog ('89, '95) and Trow ('95, '99), with the aid of improved technique, especially on the part of Trow. Hartog reaffirms the doctrine of

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De Bary, while Trow brings forward fresh evidence in behalf of a real fecundation. The rapid strides made in our knowledge of cytology by the application of better methods of technique and skill in manipulation has not only brought to light fresh questions of inquiry, but has made possible also new points of view. Consequently, the observers last mentioned find themselves differing not merely upon the old question, but upon others of deep significance in connection with the sexual process.

Following each of the two publications of Trow ('95, '99) has appeared a criticism by Hartog, in which he calls into question the statements of the former, without, however, submitting the results of any new observations. As will be shown later, the chief difference of opinion between Hartog and Trow, apart from the main contention, lies in the behavior of the nuclei during the development of the oögonium and the differentiation of the eggs. Hartog finds that, during the development of the oögonium, the nuclei fuse in groups to form the functional nuclei, one of which is present in each egg, and concludes with De Bary that no fecundation takes place. Trow finds that a certain number of the nuclei remains functional—one for each egg-cell developed—and that in certain species, as *Saprolegnia dioica* and *Achlya americana*, a real sexual process exists. Trow has not demonstrated beyond all question that fecundation does take place even in the species that seems to furnish the best evidence, but, on account of the superior methods used, we are nevertheless justified in believing that his results afford the strongest proof that has ever been advanced in favor of a sexual process, and stronger than all of his recent opponents have produced to the contrary.

Since the behavior of the nuclei is of prime importance in the differentiation of the sexual elements, and as this is one of the chief points in controversy, a somewhat detailed account of the behavior of the nuclei during the development of the oögonium and the differentiation of the egg-cells will lead the reader to a clearer understanding of the questions in debate.

The young oögonium arises as a globular enlargement at the end of a filament, into which flows dense granular cytoplasm together with a number of nuclei. With an increase in size a large vacuole appears in the base of the oögonium, and this vacuole is continuous with a cylindrical vacuole in the filament (Fig. 37, A). With further growth, which is rapid, the vacuole becomes very large and the cytoplasm is confined to a dense wall-layer. During this process a transverse wall is formed delimiting the oögonium from the filament. The nuclei, which are now distributed in the layer of cytoplasm, divide karyo-

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kinetically, thereby doubling their number, which may be ten times greater than the number of egg-cells produced in the oögonium. According to Trow the nuclei reveal a structure similar to that in the higher plants. Immediately following the division of the nuclei rapid changes take place, whose interpretation has led to differences of opinion. In both *Saprolegnia* and *Achlya*, according to Trow, only as many nuclei remain functional in the oögonium as there are egg-cells developed, the supernumerary nuclei being digested immediately after the karyokinesis mentioned above (Fig. 38, B). In *Achlya americana* the appearance of the supernumerary nuclei suggests that they may possibly divide again before disorganization. In *Saprolegnia* the same author states that some of the degenerating nuclei do really appear to unite in pairs. Hartog, on the contrary, maintains that the diminished number of nuclei was brought about by nuclear fusions, and consequently each functional nucleus remaining in the oögonium is the result of such fusions. Judging from what we now know of the behavior of nuclei in multinucleated sexual organs in which the sexual nuclei are not the product of nuclear fusions, and from the evidence which Trow has furnished, I am inclined to believe that the evidence is in favor of his conclusions, namely, that the functional nuclei of the egg-cells are not the result of fusions.

As is well known the cytoplasm now begins to ball up in masses which eventually form the egg-cells (Fig. 37, B, C). In each mass, as in the completely differentiated egg, only one functional nucleus is present. Accompanying or surrounding this nucleus is a conspicuous mass of finely granular cytoplasm, which, although appearing less highly differentiated than in certain *Peronosporeæ*, may have a similar function. The young egg rapidly becomes spherical and is provided at first with a plasma membrane only. The details in the cytoplasmic differentiation of the egg-cells have not, as yet, been critically worked out, except in so far as that is possible in the living specimen or from observations of the organs *in toto*. Whether the balling of the protoplasm described by both earlier and more recent observers is a cleavage such as is known to take place in other *Phycomycetes* can not be affirmed positively, but the facts seem to indicate a similar cleavage or a closely related process (Fig. 37, B, C).[1]

The antheridia, as is also well known, are developed from the ends of filaments which apply themselves closely to the surface of the oögonium (Fig. 37, D). When the cross-wall is formed, separating the

[1] The process of the differentiation of the egg-cells as described in the foregoing paragraph is confirmed by the very careful observations of B. M. Davis on *Saprolegnia mixta*. The manuscript of these pages had left my hands before the receipt of Professor Davis's paper.

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antheridium from the filament it contains a small but variable number of nuclei. These nuclei undergo the same changes as those in the oögonium, *i. e.*, they divide karyokinetically, and some disorganize. The fecundation-tubes are now developed and usually more than one from each antheridium. They penetrate the wall of the oögonium at

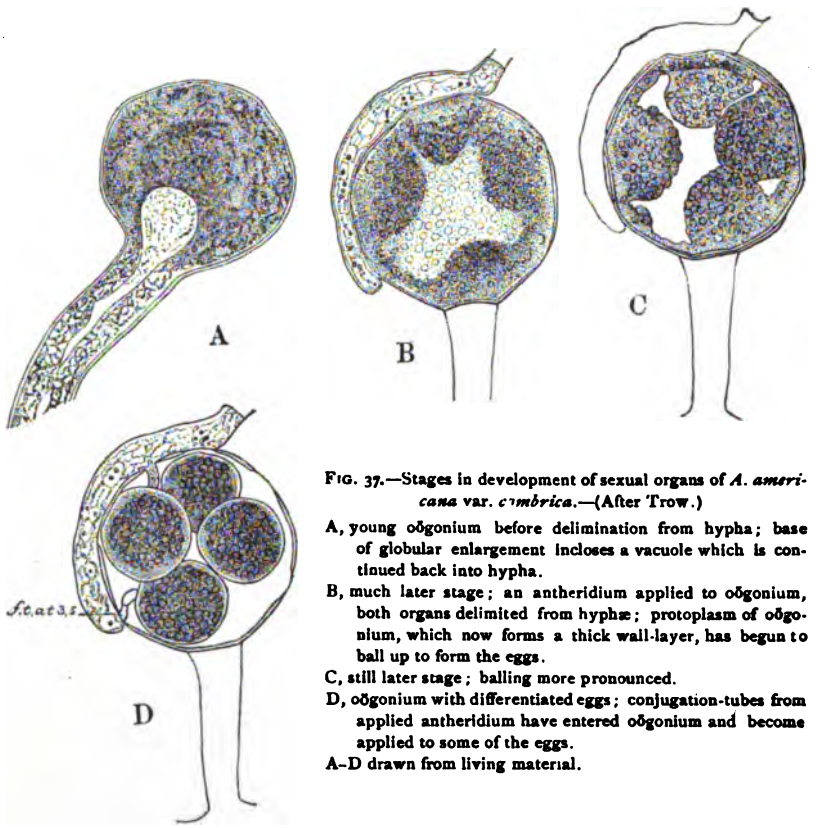

FIG. 37.—Stages in development of sexual organs of *A. americana* var. *cambrica*.—(After Trow.)

A, young oögonium before delimination from hypha; base of globular enlargement incloses a vacuole which is continued back into hypha.

B, much later stage; an antheridium applied to oögonium, both organs delimited from hyphæ; protoplasm of oögonium, which now forms a thick wall-layer, has begun to ball up to form the eggs.

C, still later stage; balling more pronounced.

D, oögonium with differentiated eggs; conjugation-tubes from applied antheridium have entered oögonium and become applied to some of the eggs.

A–D drawn from living material.

the thinner places or pits, and grow in among the eggs (Fig. 37, D, f. t.). These tubes contain nuclei which are exactly like those of the eggs, though smaller. In one case Trow was able, as he states (99, p. 159) to trace the fecundation-tube without a break into an egg which was already surrounded by a delicate membrane (Fig. 38, C). This instance "suggests that the fertilization-tube grows up to the egg, presses against it, indents it, stimulates it to the formation of a cell-wall, and grows obliquely into the mass of protoplasm, carrying at its apex a single nucleus (Fig. 38, C). . . . Later stages tend to show that the wall of the tube within the oösphere breaks down, the nucleus,

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together with a small quantity of protoplasm, is liberated, and so comes to lie in the peripheral part of the egg. The cell-wall of the oösphere is then completed, and the end of the fertilization-tube remains firmly attached to it." Although the presence of the male nucleus, while in the periphery of the egg, was not clearly demonstrated, yet this is not absolute proof to the contrary. "I have," Trow continues, "satisfied myself, however, of the presence of two nuclei in the egg *at all times* in this stage, one peripheral and one central, and the peripheral one always close to the point of attachment of the fertilization-tube."

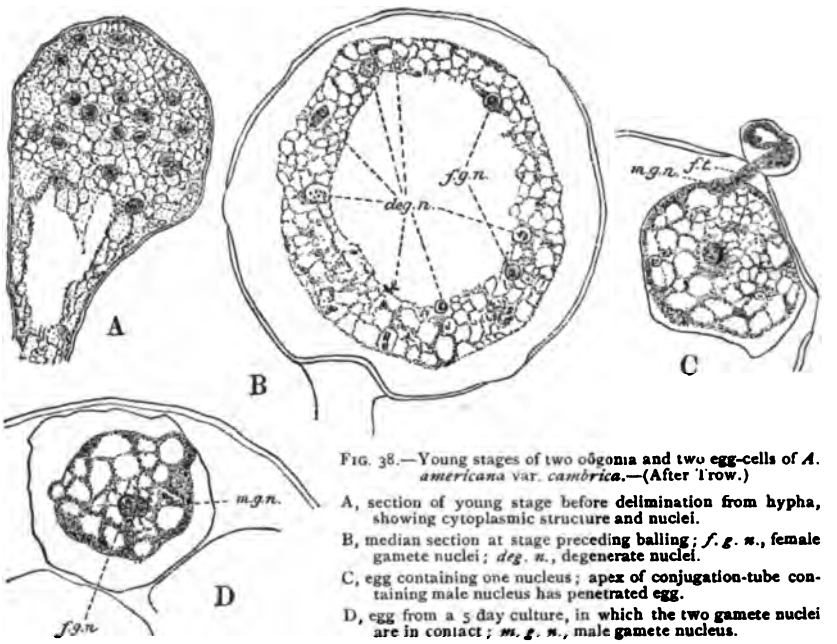

FIG. 38.—Young stages of two oögonia and two egg-cells of *A. americana* var. *cambrica*.—(After Trow.)

A, section of young stage before delimination from hypha, showing cytoplasmic structure and nuclei.

B, median section at stage preceding balling; *f. g. n.*, female gamete nuclei; *deg. n.*, degenerate nuclei.

C, egg containing one nucleus; apex of conjugation-tube containing male nucleus has penetrated egg.

D, egg from a 5 day culture, in which the two gamete nuclei are in contact; *m. g. n.*, male gamete nucleus.

At a later stage obtained from a five-day culture the two nuclei are found applied to each other in the center of the egg (Fig. 38, D). They are in the resting condition, and about the same size, the male being distinguished from the female only by its smaller nucleolus. From the fact that the sexual nuclei were found side by side in a five-day culture, and from an examination of many hundreds of oöspores from six- to eight-day cultures, it is inferred that about three days are required for the complete fusion, during which time the nuclei remain in the resting condition, a phenomenon of frequent occurrence among thallophytes. In the oöspores of nine- or ten-day cultures, which have developed a well-differentiated cell-wall, only one nucleus was observed. Later, during germination, the fusion nucleus divides karyo-

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kinetically, and the process is repeated until by the time a germ-tube is evident, or even before, about twenty nuclei are present.

It may be objected that Trow's evidence of the passage of the sperm nucleus into the egg is insufficient, and that the two nuclei seen in the young oöspore may have been derived from a division of the unfecundated egg-nucleus. While such objections have but little weight, yet we must admit that the possibility of their truth is not excluded. For many of us Trow's observations will have a probability bordering on certainty. Although the conclusions of Trow require confirmation, yet I think it can be fairly said, and that too with all due respect for the ability and skill of De Bary and others whose observations tend to confirm his view, that Trow has furnished the strongest evidence that has thus far been brought forward in support of the existence of sexuality in certain species of the Saprolegniaceæ.

From the foregoing it is clear that certain similarities exist between these genera and such forms as *Albugo*. The development of the sexual organs themselves, and the earlier conduct of the numerous nuclei which enter the young sexual organs from the parent hyphæ, are quite parallel. The great difference lies in the differentiation of the egg-cells. In *Saprolegnia* and *Achlya* we have developed, as a rule, several eggs, and there is no trace of periplasm. The supernumerary nuclei disorganize before the egg-cells are differentiated. In *Albugo* and closely related genera, the supernumerary nuclei, if we may be permitted to speak of those of the periplasm as such, having different and additional functions, disappear later.

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CHAPTER V.—TYPE OF THE ASCOMYCETES AND RHODOPHYCEÆ.

Within recent years our knowledge of the sexual process in certain of the higher fungi, the *Ascomycetes*, has been greatly advanced by the classical researches of Harper. These researches have inaugurated a sort of renaissance in the study of the sexual process in the fungi; for within the last decade the doctrine of sexuality in the *Ascomycetes* as advanced by De Bary has been strenuously denied in some quarters, especially among the mycologists of the Brefeldian school, and the view that no sexual reproduction at all occurs in this group had gained considerable ground.

Harper's work upon certain *Perisporeaceæ* and *Discomycetes* leave no doubt concerning the true sexual process in those groups, and it is reasonable to expect that further research will bring to light the presence of sexual reproduction in other genera in which the existence of sexuality seems far more questionable.

In the development of the sexual organs and in the behavior of the egg-cell, there is represented here a type of sexual reproduction very different from that known in other fungi and in the green algæ. The closest parallel is found in the *Rhodophyceæ* and in certain lichens. There is certainly a striking and suggestive resemblance between the structure of the sexual organs and the process of development subsequent to fecundation in *Sphærotheca*, *Pyronema* and *Collema* on the one hand, and in such forms of the red algæ as *Batrachospermum* and *Nemalion* on the other. It is not improbable that further research will reveal a tolerably well connected series from forms like *Sphærotheca* to the remarkably complex *Dudresnya*, and we may accept without much reserve the view that the great groups to which these representatives belong represent related phylogenetic series. In this chapter, therefore, I shall present the sexual process in *Sphærotheca*, *Pyronema*, *Collema*, *Batrachospermum* and *Dudresnya* as representative of the type of sexuality in the Ascomycetes, including that form in lichens, and in the Florideæ.

What follows concerning *Sphærotheca* and *Pyronema* is based exclusively upon the studies of Harper ('95, '96, 1900).

SPHÆROTHECA.

Both antheridia and oögonia of *Sphærotheca* arise as lateral branches of neighboring mycelial filaments, the development of the oögonium

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preceding somewhat that of the antheridium. Each consists at first of a short oval branch, which is distinguished from the ordinary vegetative hyphæ only by its denser protoplasmic contents and by standing at right angles to the surface of the leaf of the host plant.

As soon as the oögonium has attained a length equal to two or three times its width, and a diameter which is about twice that of a mycelial filament, it is cut off from the parent hypha by a cross-wall. At this stage it possesses a single nucleus which can scarcely be distinguished from the nuclei of vegetative cells. Frequently, before the young oögonium is delimited by the cross-wall, the antheridial branch appears quite near the base of the former, and grows upward, closely applied to its side (Fig. 39, A). The oögonium appears to grow faster than the antheridial branch at first, thereby bending over toward the latter,

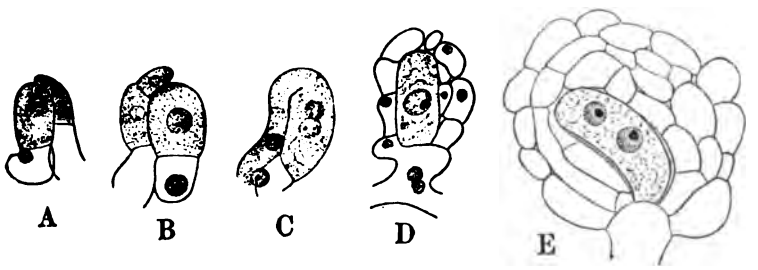

FIG. 39.—Sexual organs and fecundation in *Sphærotheca castagnei* Lm.—(After Harper.)

A, young oögonial and antheridial branch, oögonium on left.
B, later stage of same, antheridium delimited by a transverse wall.
C, copulation of antheridium and oögonium; the two sexual nuclei in contact in oögonium.
D, oögonium in which the sexual nuclei have fused.
E, young ascogonium with two nuclei; wall of perithecium is now several cells in thickness.

and giving the impression that the contiguous walls were grown together, and that the growth of the oögonium was retarded on the side next the antheridium. The antheridial branch is now separated from its mycelial filament by a cross-wall which is higher in position than the corresponding wall of the oögonium. This cell contains also only one nucleus. When the development of the oögonium is complete the antheridial branch elongates and its nucleus divides. One of the resulting daughter-nuclei passes into the somewhat attenuated end of the cell, which is cut off from the lower part to form the antheridium (Fig. 39, B). While the stalk cell now elongates and the antheridium increases in size the oögonium experiences little change; consequently, the antheridium is carried upward, and finally comes to lie as a cap placed more or less obliquely on the top of the oögonium. At this stage the nucleus of the egg-cell is larger than the ordinary

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vegetative nuclei, while that of the antheridium is correspondingly smaller.

The cell-walls between the antheridium and oögonium are dissolved, the male nucleus passes through the opening thus formed into the oögonium, wanders toward the egg-nucleus, and soon fuses with it (Fig. 39, C). After the entrance of the male nucleus the antheridium still remains filled with cytoplasm which is in direct communication with the cytoplasm of the oögonium. Very soon, however, the opening between the two organs is closed by a new wall, when only a small quantity of cytoplasm is to be seen in the antheridium.

Immediately after fecundation the oögonium begins a steady growth. The egg-cell does not round off by means of self-plasmolysis either before or after fecundation, thereby becoming separated from the

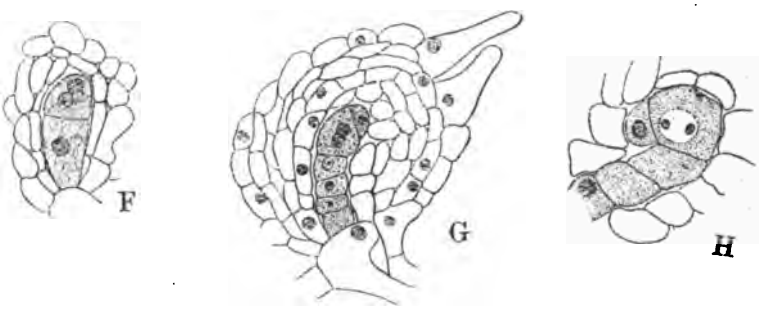

FIG. 40.—Development of ascogonium of *Sphærotheca castagni*.—(After Harper.)
F, ascogonium with two cells; upper cell has two nuclei.
G, mature ascogonium; the penultimate, or ascogenous cell, contains two nuclei.
H, the two nuclei in the young ascus have fused, fusion nucleus containing two nucleoli.

wall of the oögonium. In this respect the *Ascomycetes* differ from all other plants except the *Rhodophyceæ* with which they form a striking parallel.

A few steps further in the development of the fecundated egg will be traced to show the relation in the course of development of the fusion of the sexual nuclei to the vegetative nuclear fusion occurring in the young ascus. In speaking of this part of the development the term ascogonium will be used.

A series of nuclear and cell-divisions now follow in the developing ascogonium, so that ultimately a row of five or six broad cells result (Fig. 39, D, E, and Fig. 40, F, G). Nuclear and cell-division are not dependent upon each other, and they do not seem to follow in the same order. In different stages of this growth, from one to three nuclei are to be seen in the distal cell of the ascogonium, but when the definite number of cells is formed two nuclei are always to be found

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in the penultimate cell of the row, while all other cells of the ascogonium are uninuclear (Fig. 40, G). This penultimate cell becomes the ascus; it is not to be regarded as the exact equivalent of any other cell of the ascogonium, and its two nuclei are not necessarily sister nuclei, for before the last cross-wall is formed in the ascogonium the distal cell may contain three nuclei, and of these any pair may remain in the penultimate cell. With further development these two nuclei fuse (Fig. 40, G, H). This fusion is comparable to the nuclear fusion occurring generally in young asci, and consequently it has not the significance of fecundation, but represents merely a vegetative union. In this connection it may be mentioned that the objections which Dangeard ('97) has raised against the true sexual process described by Harper do not seem to me to merit any serious consideration.

Sphærotheca represents one of the simplest and perhaps the most primitive forms of the true *Ascomycetes*, especially as regards the development of the ascogonium. In *Erysiphe* and *Ascobolus* a greater complexity in the development of the ascogonium obtains, but there can be no doubt as to the nature of their sexual organs and the fusion of their true sexual nuclei, especially in *Erysiphe*.[1]

PYRONEMA.

In *Pyronema* we have a form which possesses for us a twofold interest. I refer to the trichogyne-like organ borne by the oögonium and the multiple fecundation, or the fusion in pairs of two or more male with two or more female nuclei in the oögonium.

The development of the sexual organs is briefly as follows: The cells of the mycelium from which these organs are developed are multinucleate. Both oögonia and antheridia arise from the apical cells of thick hyphal branches, standing vertical to the substratum. The young oögonium is more spherical and can be distinguished from the young club-shaped antheridium standing by its side. Soon a small papilla appears at the apex of the oögonium, which eventually becomes the conjugating-tube or trichogyne (Fig. 41, A. B). Both organs are multinucleate from the start, the number of nuclei increasing by division as the cells grow in size. "The nuclear multiplication, however, is out of proportion to the vegetative growth, so that when the sexual cells are mature they contain relatively to their size more nuclei than do the ordinary vegetative mycelial cells" (Harper, 1900, p. 341). A broad stalk-cell is cut off from the base of the oögonium at a relatively late stage in its development,

[1] For a detailed discussion of these processes and the phylogenetic significance of the ascus fruit, the reader is referred to the original papers of Professor Harper ('95, '96, 1900).

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and a number of stalk-cells is usually to be distinguished at the base of the antheridium. With further development the papilla or young conjugating-tube elongates rapidly, its tip curving somewhat to meet the end of the club-shaped antheridium which curves slightly over the oögonium, frequently exceeding the latter in height (Fig. 41, C). At first the contents of the trichogyne and the oögonium are continuous (Fig. 41, B). It is multinucleate, and the nuclei do not appear to be different from those of the oögonium. Long before the trichogyne becomes fused with the antheridium, a cross-wall is formed

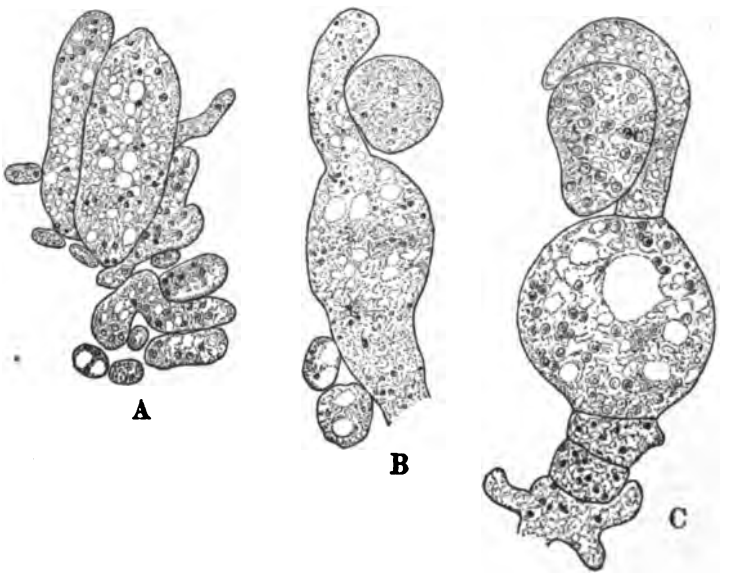

FIG. 41.—Development of sexual organs in *Pyronema confluens*.—(After Harper.)

A, young pair of sexual organs with several vegetative cells below.
B, older pair, trichogyne not yet delimited from oögonium; antheridium cut transversely, hence seen in transverse section.
C, oögonium and antheridium in longitudinal section; oögonium stalk with budding vegetative hyphæ; trichogyne with hyaline beak, its nuclei swollen and transparent.

at the juncture of the tube with the oögonium, delimiting it from the latter. This wall is formed before the sexual cells or the trichogyne have reached their mature size. Whether nuclear divisions occur in the tube after it is cut off was not determined (Fig. 41, C). During subsequent growth the nuclei in the trichogyne do not increase in size as do those of the antheridium and oögonium, but sooner or later show signs of disintegration. They swell up without an increase of their contents until they may equal in size the sexual nuclei, but they are very transparent. Later, during the formation of the fusion-pore

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between the trichogyne and the antheridium, these nuclei collapse and break down into dense strands or shreds, which are frequently so connected as to form a coarse and much broken network in the cytoplasm (Fig. 42, D). The structure of the mature sexual organs, which are aggregated in rosette-like clusters, is summarized by Harper as follows (1900, p. 344):

> The oögonium is a spherical or flask-shaped cell filled with dense protoplasm and many nuclei, which are very much larger than those of the ordinary vegetative cells. Its stalk consists of two or three broad disk-shaped cells, of which the basal one is a part of the mass of thickened, swollen cells forming the base of the rosette. The apex of the oögonium is continued into the narrow conjugating tube which curves upward to unite with the end of the antheridium. The antheridium is a curved, club-shaped cell, thickest near its upper end, and tapering gradually to its base, where it is continued into a stalk of one or more cells. The basal wall of the antheridium is, as a rule, somewhat higher up than that of the oögonium. It follows a somewhat oblique path upward, conforming rather closely to the surface of the oögonium, and its apex is even with, or reaches somewhat past, that of the latter.

The mutual relation of the sexual organs will be best understood from Fig. 44.

The changes taking place in the mature sexual apparatus, and which lead up to fecundation, are of much interest, especially when compared with the same process in other plants exhibiting similar phenomena. First among these are what may be termed the receptive spots of both the antheridium and the trichogyne. In that part of the antheridium near which the tip of the trichogyne presses against its wall and where the fusion-pore is formed, an area of protoplasm is differentiated as a finely granular and irregularly lens-shaped disk from which the nuclei have withdrawn. This granular area, although situated in the antheridium, Harper very fittingly compares to the so-called mouth-piece, or receptive spot, of the egg in such algæ as *Œdogonium* and *Vaucheria*. The beak-like prolongation of the trichogyne reveals also a similar, though less conspicuous, cytoplasmic differentiation (Fig. 41, C; Fig. 42, D). These areas seem to exercise a chemotactic influence which tends to bring together the tube and the antheridium, and also to secrete an enzyme by which the walls are dissolved in the formation of the conjugation-pore. The presence of a similar differentiation in both the tube and the antheridium would seem to indicate also that the influence is mutual.

At the point where the beak of the trichogyne is closely pressed against the antheridium the walls are dissolved and a pore is formed by which the cytoplasm of these two cells is made continuous (Fig.

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42, D). During this process, or sometimes later, as stated in a preceding paragraph, the nuclei of the trichogyne disintegrate. When this has taken place the antheridial nuclei begin to migrate through the pore into the trichogyne, whose protoplasmic contents become still further disorganized. This migration of nuclei continues until the tube is quite densely filled and sometimes slightly swollen (Fig. 42, E).

In the meantime conspicuous changes have been taking place in the oögonium. The nuclei, which are evenly distributed throughout the interior of this organ, begin to migrate toward the center, where they

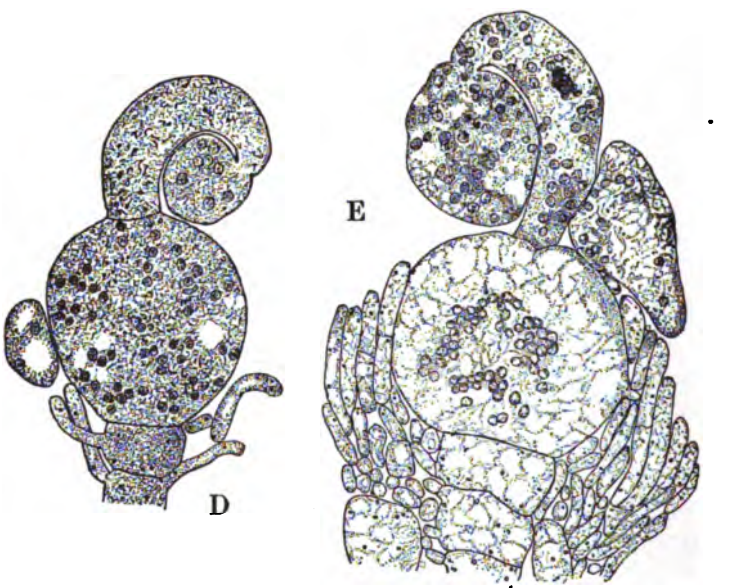

FIG. 42.—Copulation of sexual organs of *Pyronema*.—(After Harper.)

D, the trichogyne has copulated with antheridium, nuclei in trichogyne disintegrated; hypothecial hyphæ springing from stalk cells.

E, trichogyne filled with nuclei from antheridium; antheridium curved around trichogyne so that the latter appears in section to cut through it; trichogyne still separated from oögonium by transverse wall.

become collected into a dense, hollow sphere, equal in diameter to about half that of the oögonium, or they may aggregate into an irregular, crescent-shaped mass in either the upper or lower half of the oögonium (Fig. 42, E). Less frequently several masses of nuclei are formed instead of one.

The cytoplasm of the oögonium, which was charged with densely staining substances, becomes tenuous and loosely spongy in texture. After the oögonial nuclei have aggregated in the center of the egg-cell, the basal wall of the trichogyne breaks down and the antheridial nuclei

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pass at once into the oögonium, to the central mass of egg-nuclei, and become mingled with them (Fig. 43, F). The number of male nuclei entering the oögonium does not seem to be exactly the same as the number of egg-nuclei to be fecundated. Both sexual organs arise as multinucleate cells, and, as there is no evidence subsequently of a parallel series of nuclear divisions in each, it is difficult to see how exactly the same number could be provided in each organ.

Only a small portion of the cytoplasm of the antheridium passes into the egg-cell, so that here, as elsewhere in the plant kingdom, the superior significance of the nuclei in fecundation is strikingly manifested. The male and female nuclei mingled in the central group are indistinguishable in size, structure, and staining qualities, so that it is

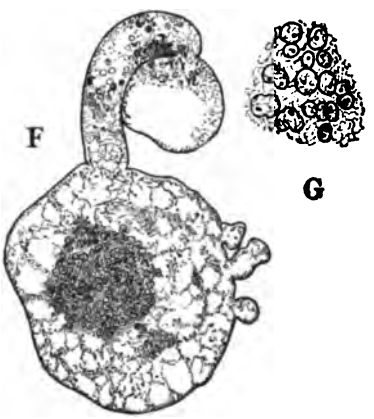

FIG 43.—Fusion of sexual nuclei in the oögonium of *Pyronema*.—(After Harper.)
F, basal wall of trichogyne dissolved, male and female nuclei collected in dense mass in center of oögonium and fusing in pairs; nuclei still present in trichogyne and upper end of antheridium; ascogenous hyphæ budding out from oögonium.
G, group of fusing nuclei from central mass of nuclei in an oögonium.

impossible to pick out a single nucleus and say whether it has come from the oögonium or antheridium. The nuclei fuse in pairs while they are aggregated in the dense mass (Fig. 43, G). The behavior of all the nuclei in the center of the mass was not determined with certainty, but there is every reason to believe that the rule of fusion in pairs holds for nearly the whole mass. Harper expressly states that there is no general fusion of the nuclei into a single mass, as can be clearly seen when the nuclei scatter after fusion.

The oögonium of *Pyronema* functions at once as an ascogonium. All fecundated nuclei pass into ascogenous hyphæ and may reach the asci. Here the young ascus develops also from the penultimate cell of a bent ascogenous hypha, and in it two nuclei are present which

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fuse, but this fusion does not, as previously stated for *Sphærotheca*, represent a sexual process.

It will thus be seen that the process of fecundation in *Pyronema* consists in the union of multinucleated gametes and in the fusion of their nuclei respectively in pairs. Here as in all other plants, whether possessing uninuclear or multinuclear gametes, the fact of prime importance is the fusion of the sexual nuclei, the cytoplasm playing perhaps an incidental and secondary rôle. The fusion of numerous pairs of sexual nuclei in the egg-cell is after all not so remarkable since the significance and final result is the same as in the case of uninuclear gametes. We may upon strong grounds conclude with Harper that "this aggregation of nuclei at the time of fertilization seems to be simply a provision for the pairing of male and female nuclei with the

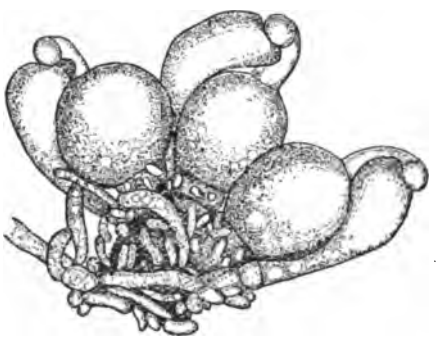

FIG. 44.—Group of 3 pairs of sexual organs of *Pyronema* in surface view.—(After Harper.)

greatest certainty and despatch." The cell, considered as a morphological and physiological unit, is just the same no matter whether it possesses one or many nuclei, and in this respect there seems to be no good reason for regarding a "cœnocyte" as a tissue.

BATRACHOSPERMUM.

As representing the sexual process in the *Rhodophyceæ* I have selected *Batrachospermum* and *Dudresnya*. *Batrachospermum* is selected on account of the comparative simplicity of the spore-fruit development, and because the fusion of the sexual nuclei, as observed by Osterhout (1900), leaves not the slightest doubt as to the exact nature of a sexual reproduction. The classical object, *Dudresnya*, affords an illustration of a complex series of phenomena following fecundation, which has, until recently, been regarded as representing several separate sexual acts.

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With the process of fecundation as the primary object in view, *Batrachospermum* has been recently studied by Davis ('96), Schmidle ('99), and Osterhout (1900). As regards the cytological details bearing upon fecundation the work of Osterhout seems to have been the most thorough.

The well known female sexual organ, the carpogonium, of *Batrachospermum*, is a single cell consisting of a somewhat flask-shaped basal part, the trichophore, in which is the egg-nucleus, connected by a narrow neck to the elongated, cylindrical or club-shaped, upper part, the trichogyne (Fig. 45, B). In *B. boryanum* Sirodot, the species studied by Osterhout, the chromatophore of the trichophore is continued into the trichogyne. The structure of the nucleus is the same as that of higher plants. The spermatia are globular cells with one nucleus and a reduced chromatophore in younger stages (Fig. 45, A).

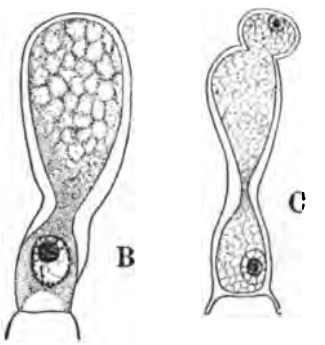

FIG. 45.—Sexual organs of *Batrachospermum boryanum* Sirodot.—(After Osterhout.)

A, antheridium with one nucleus and vacuolate cytoplasm.
B, mature carpogonium before fecundation.
C, spermatium has copulated with trichogyne of carpogonium; cytoplasmic fusion has taken place, but nucleus is still in spermatium; egg-nucleus lies in trichophore.

Schmidle ('99), whose observations were made chiefly upon *B. bohneri*, agrees with Osterhout as regards the structure of the carpogonium, but in the spermatia of this species he finds, almost invariably, two nuclei. Davis ('96), differing from both Schmidle and Osterhout, claims that in *B. moniliforme* Roth., *B. cærulescens* Sirodot, and *B. boryanum*, the trichogyne is a distinct cell possessing a well defined nucleus and chromophore, and connected with the trichophore by a strand of protoplasm. The methods used by Davis at the time were inadequate for the better differentiation of the nucleus, and his conclusion is in all probability incorrect.

The copulation of the spermatia with the trichogyne and the fusion of the sexual nuclei is as follows: One to several spermatia, which are now provided with a cell-wall, become attached to the trichogyne chiefly near the end (Fig. 45, C, and Fig. 46, D, E). After the dissolution of the cell-membranes at the point of contact the nucleus of the spermatium enters the trichogyne and passes down through it into the base of the carpogonium. The canal between the trichogyne and the

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basal part of the carpogonium now becomes narrower and is finally closed by the swelling or growth of the cell-wall, so that the entrance of other male nuclei is impossible (Fig. 46, D). In case other male nuclei enter the trichogyne from other adhering spermatia, as frequently happens, these fragment and disappear, and the same fate befalls those nuclei that remain in other adhering spermatia. Soon after the male nucleus enters the trichophore it fuses completely with the egg-nucleus (Fig. 46, D, E). This fact, so unmistakably observed by Osterhout,

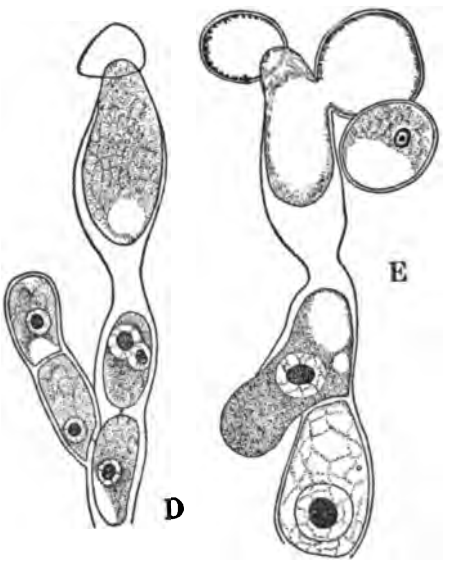

FIG. 46.—Fusion of sexual nuclei and immediate subsequent development of fecundated egg in *Batrachospermum*.—(After Osterhout.)

D, sexual nuclei in act of fusing; an empty spermatium adheres to tip of trichogyne.

E, later stage; the trichophore has increased in size and sent out a protuberance; the empty spermatium which has copulated with the trichogyne has furnished the male nucleus; below it is a spermatium with a nucleus, and above to the left is one in which the nucleus has undergone fragmentation.

leaves no room for doubting the existence of a true fecundation in *Batrachospermum*.

Schmidle did not observe the actual fusion of the sexual nuclei, but he concludes that the same takes place. He asserts that, together with the two nuclei which he finds in the spermatium, a portion of the cytoplasm also enters the trichogyne, while the plasma membrane remains behind, save in exceptional cases in which the spermatia were quite empty. Davis ('96) having failed to observe the entrance of the male nucleus into the egg-cell, inclined to the view that only cytoplasmic contact was necessary in *Batrachospermum* to insure the further

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development of the spore fruit from the egg-cell. Such a doctrine has, of course, the value of mere conjecture only.

The fusion nucleus increases in size and shows clearly a single large nucleolus and a well-defined threadwork in which are held distinct chromatin granules. The trichophore now begins to send out one or more protuberances (Fig. 46, E). The fusion-nucleus divides, and one of the daughter-nuclei passes into a protuberance which is then cut off by a transverse wall. By a repetition of this process many cells are produced, each containing a nucleus which is a descendant of the fusion-nucleus. Each of the cells thus borne by the carpogonium will give rise to gonemoblast filaments, whose end cells form the carpospores.

DUDRESNYA.

From the foregoing it will be seen that the sexual process and the subsequent development of the fecundated egg in *Batrachospermum* are comparatively simple, but in the vast majority of the *Rhodophyceæ*, because of the peculiar structure of the thallus, the details in these processes are extremely difficult to follow even in the most favorable cases.

In the better known representatives, such as *Dudresnya* and the simpler *Callithamnion*, the carpogonium does not give rise to the spore fruit (cystocarp), as in *Nemalion* (Wille) and *Batrachospermum*, but from each carpogonium whose egg-cell has been fecundated a number of filaments (two or three in *Dudresnya*) are developed, which fuse with certain vegetative cells, and from which, in connection with a part of the filament, the cystocarps are developed. These filaments are the *oöblastema filaments* of Schmitz ('83) and the *sporogenous filaments* of Oltmanns ('98). The vegetative cells with which these fuse are known as *auxiliary* cells or brood cells. This fusion of the sporogenous filaments with auxiliary or brood cells was regarded by Schmitz and his followers as a second fecundation, a phenomenon unparalleled among plants, and which, as Schmitz put it, was contrary to all tradition: "Einen zweimaligen Befruchtungsact im Entwickelungskreise einer einzelnen Species anzunehmen, dagegen sträubt sich zur zeit die botanische Anschauung vollständiger, das widerspricht aller Tradition."

The recent researches of Oltmanns ('98) seem to show what is, in all probability, the true significance of the fusion of sporogenous filaments and auxiliary cells. He maintains that the fusion of the sporogenous filament, or a cell of the same, and an auxiliary cell is not a sexual process, since it is only a cytoplasmic and not a nuclear fusion that takes place. Furthermore, the nuclei of the carpospores, as in

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Batrachospermum, are the descendants of the fusion nucleus, resulting from the union of male and female nuclei in the carpogonium. The nuclei of the auxiliary cells never take a morphological part in the formation of the carpospores. According to this view, therefore, the auxiliary cells are merely brood cells, their fusion with the cells of the sporogenous filaments representing a peculiar condition of nutri-

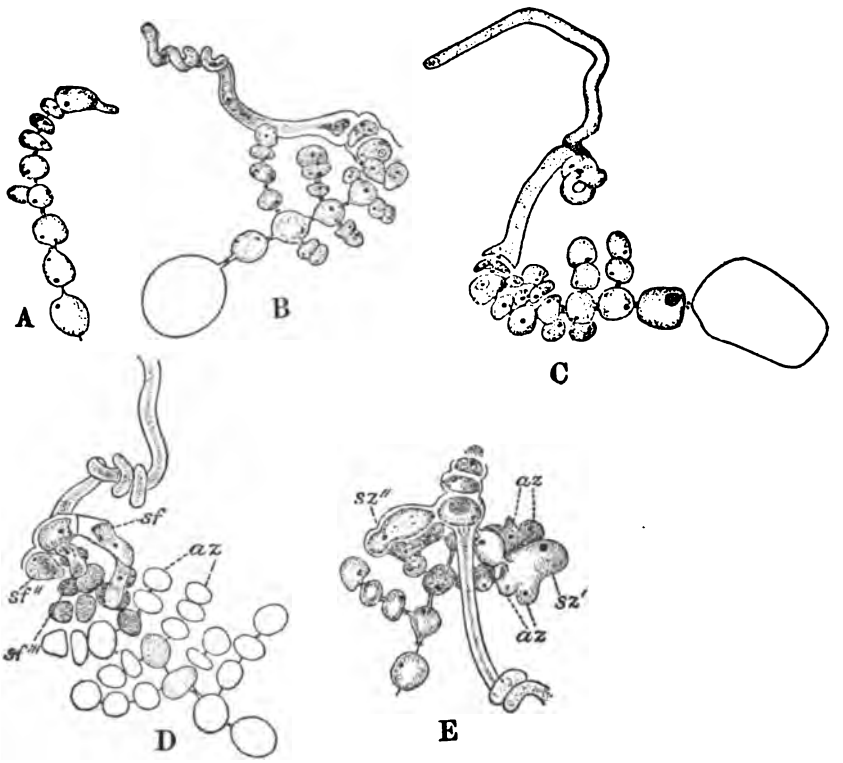

FIG. 47.—Carpogonium and its development subsequent to fecundation in *Dudresnya purpurifera*.—(After Oltmanns.)

A, carpogonial branch with young carpogone at upper end.
B, later stage; nucleus of carpogonium lies some distance up in trichogyne, whose end is twisted.
C, still later stage; nucleus lies in coiled part of trichogyne.
D, carpogonial branch after fecundation; fusion of sporogenous filaments *sf* with auxiliary cells; *as*, auxiliary cells.
E, later stage; *ss*, sporogenous cells; *as*, auxiliary cells.

tion. A further discussion of this phenomenon is reserved for a later paragraph.

In order to comprehend fully the statements of the preceding paragraphs, it will be necessary to follow somewhat in detail the process involved in one of the forms referred to, as, for example, *Dudresnya*.

In *Dudresnya purpurifera*, according to Oltmanns ('98), the car-

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pogonium is developed from the end cell of a short branch (Fig. 47, A), whose remaining cells give rise to numerous side branches (Fig. 47, B). The end cells of these side branches may become auxiliary cells. The trichogyne is unusually long, showing spiral-like turns either at its middle or nearer the base. The nucleus lies in the ventral part of the young carpogonium. Later it passes up into the trichogyne, and when the carpogonium is ready for fecundation, the nucleus is to be found in the coiled region of the trichogyne (Fig. 47, C). The spermatium applies itself to the tip of the trichogyne, which projects slightly beyond the general surface of the thallus. The cell-walls at the point of contact are dissolved, the sperm-nucleus passes down into the trichogyne and fuses with the egg-nucleus in a manner described by Wille ('94) for *Nemalion*.

Oltmanns did not observe the actual fusion of the sexual nuclei in *Dudresnya*, but in repeated instances two nuclei were seen lying tolerably near each other in the trichogyne, and at a later stage a single nucleus was found in the ventral part of the carpogonium, which he regarded as the fusion-nucleus. The union was observed, however, in *Dasya elegans*, and personally Oltmanns believes the fusion in *Dudresnya* to be too probable to justify an exhaustive study. It may be remarked that in general this is by no means a safe principle to follow.

After fecundation the base of the carpogonium (or shall we say the fecundated egg-cell) segments into cells which increase in size and begin to grow into sporogenous filaments. In *Dudresnya purpurifera* two or three of such filaments arise from the carpogonium, one on either side, with sometimes a third between them (Fig. 47, D, *sf*). The sporogenous filaments, which soon become segmented into cells by transverse walls, grow downward among the lateral branches of the carpogonial branch and fuse with some of the end cells of these branches, which have become auxiliary cells (Fig. 47, E, *az*). Certain cells of the carpogonial branch may function also as auxiliary cells. The auxiliary cells are distinguished by their form and denser protoplasmic contents. Usually only one cell of a sporogenous filament unites with an auxiliary cell or cells.

The filaments continue their growth in length, fusing with other auxiliary cells which may be borne upon other and widely separated vegetative branches (Fig. 49). The fusion of any auxiliary cell with that of a sporogenous filament represents only a cytoplasmic fusion and not a sexual act. This process with the immediate subsequent changes is briefly as follows: As soon as the cell-walls at the point

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of contact dissolve, the cytoplasm of the two cells becomes continuous. The nuclei show no tendency even to approach each other, but, on the contrary, that of the cell of the sporogenous filament seems to repel the nucleus of the auxiliary cell, as this one generally retreats from its former central position to the side farthest removed from the point of contact of the two cells (Fig. 47, E, and Fig. 48, A). That part, or half, of the fusion cell which corresponds to the sporogenous filament now begins to send out a protuberance into which the sporogenous nucleus and dense cytoplasm pass (Fig. 47, E, *sz*). In the earlier developmental stages following fecundation this protuberance develops an additional branch of the sporogenous filament which is to seek and fuse with other auxiliary cells (Fig. 48, A, B). In case of the development of a cystocarp from the fusion cell, the protuberance in question, after the division of its nucleus, will be cut off as a rounded cell (Fig. 48, C), which will give rise ultimately to a spore fruit.

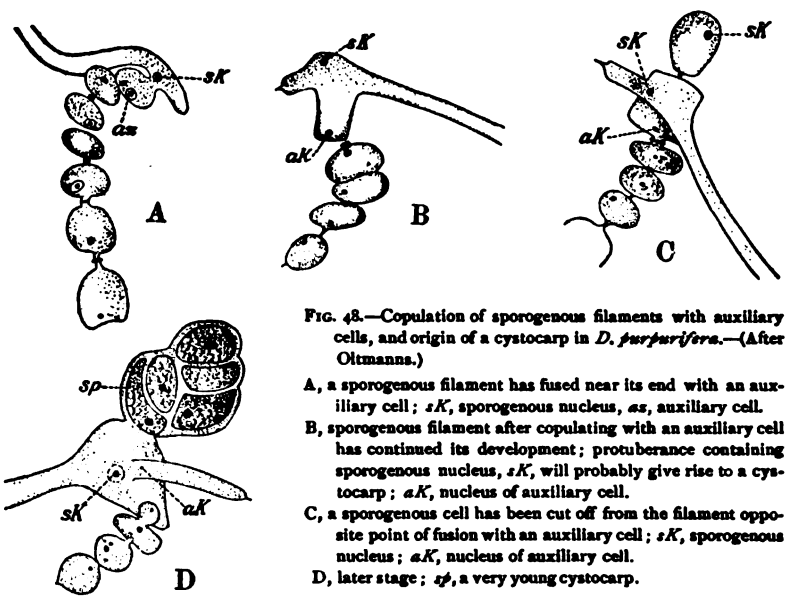

FIG. 48.—Copulation of sporogenous filaments with auxiliary cells, and origin of a cystocarp in *D. purpurifera*.—(After Oltmanns.)

A, a sporogenous filament has fused near its end with an auxiliary cell; *sK*, sporogenous nucleus, *as*, auxiliary cell.

B, sporogenous filament after copulating with an auxiliary cell has continued its development; protuberance containing sporogenous nucleus, *sK*, will probably give rise to a cystocarp; *aK*, nucleus of auxiliary cell.

C, a sporogenous cell has been cut off from the filament opposite point of fusion with an auxiliary cell; *sK*, sporogenous nucleus; *aK*, nucleus of auxiliary cell.

D, later stage; *sp*, a very young cystocarp.

In *Dudresnya purpurifera* the nuclei of auxiliary cells which have fused with cells of the sporogenous filaments tend to diminish in size and disappear, while in *D. coccinea* the nucleus of the auxiliary cell may remain normal and divide. In no case, however, do these auxiliary nuclei show any disposition to fuse with a sporogenous nucleus.

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The development of the sporogenous filaments, their fusion with auxiliary cells, and the origin of cystocarps from the fusion cells will be more readily understood from the diagram in Fig. 49. At *a*, after

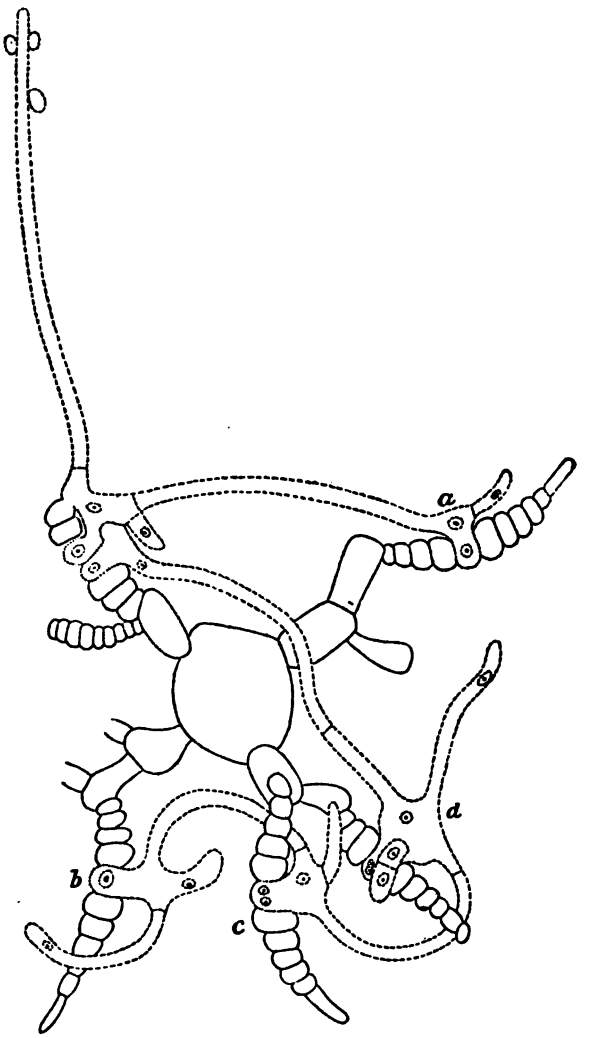

FIG. 49.—Diagram showing origin of sporogenous filaments and their union with various auxiliary cells in *Dudresnya coccinea*; parts drawn by means of short dashes indicate trichogyne and sporogenous filaments, while the dots indicate the auxiliary cells; *a*, *b*, *c*, *d*, places where sporogenous filaments have united with auxiliary cells.—(After Oltmanns.)

the sporogenous filament had fused with the auxiliary cell, the sporogenous nucleus divided, one daughter-nucleus remaining in the fusion cell, the other passing into the end of the filament which is cut off by

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a transverse wall. This end cell continues the development of the sporogenous filament, which in turn may fuse with other auxiliary cells. At *b*, *c*, *d* the sporogenous parts of the fusion cells have given rise to branches which will produce either sporogenous filaments or spore fruits, as shown in Fig. 48, C, D.

In all authentically known cases among the *Rhodophyceæ* the structure of the female sexual organ, the carpogonium, or we may say the oögonium, and the process of fecundation is essentially the same, but the development of the cystocarps from the fecundated egg differs widely in detail among the various genera.[1] So far as is known the sporogenous filaments reach their highest development and complexity in *Dudresnya*, in which, as we have seen, the fusion of each of the sporogenous filaments takes place with a greater number of widely separated auxiliary cells. In other forms, such as *Callithamnion* and *Dasya* (Oltmanns, '98), in which only one or two closely situated auxiliary cells take part in the formation of the cystocarps, the sporogenous filaments may consist of only a few cells at most. In these cases we can scarcely speak of sporogenous filaments, but rather of sporogenous cells.[2]

The relation which an auxiliary and a sporogenous cell sustain to each other is somewhat different in the several known genera. As already stated for *Dudresnya*, the sporogenous part of the fusion cell (Fig. 48, B, C, D) gives rise to the cystocarp, while in *Glœosiphonia capillaris* (Oltmanns, '98) the sporogenous cell, after the fusion of its contents with the auxiliary cell, may take no further part in the development. Its cytoplasm and nucleus pass into the auxiliary cell, and a cell-wall is formed separating the old cavity of the sporogenous cell from the auxiliary cell. From the auxiliary cell the cystocarp is now developed. A similar process takes place also in *Callithamnion* and *Dasya*. Although the behavior of the two cells in the last two genera named suggests a greater similarity to a real fecundation than in *Dudresnya*, yet the nuclei of the two cells never fuse. The sporogenous cell merely leaves its original abiding place to take possession of the auxiliary cell, using it as a basis from which to develop the spore fruit; for the nuclei of the auxiliary cell either disappear, or, if they persist, take no part in spore-fruit formation. The nuclei of all the cells of the spore fruit are descendants of the sporogenous nuclei, and are therefore sporophytic nuclei, while those of the auxiliary cells are gametophytic. The process occurring in

[1] In addition to the authors mentioned above see also Philips, '95, '96, '97, '98. Osterhout, '96. Hassenkamp, '00.

[2] See Oltmanns, '98, Taf. VII, Figs. 11–20.

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Callithamnion, *Glœosiphonia*, *Dasya* and others is after all not so extraordinary as it may at first appear, since the superior significance of the nucleus in all constructive metabolism of the cell has been thoroughly demonstrated.

If the doctrine of Oltmanns be correct, and the facts seem to justify his conclusion, we have in the sporogenous filaments of *Dudresnya* and similar genera of the *Rhodophyceæ* a *sporophyte*, which, for the purpose of nutrition, fuses with auxiliary cells, and, because of the better nutrition, is capable of producing several spore fruits. The auxiliary cells must, therefore, be regarded merely as special brood cells, their fusion with the cells of the sporogenous filaments being homologous with the fusion of vegetative cells.

As regards the existence of an alternation of generations in the *Rhodophyceæ*, there still remains the question upon which De Bary laid some stress, namely, that in the *Rhodophyceæ*, as well as in the *Ascomycetes*, there is no rounding up or separation of the egg as an independent cell in the oögonium, such as occurs, for example, in *Coleochæte*, in the *Bryophyta* and *Pteridophyta*. In the second place the determination of the number of chromosomes in these generations and the point in the life-cycle at which the numerical reduction of the chromosomes takes place are factors, which, in the light of important existing theories, must be taken into consideration. The first of these questions may be of comparatively little importance, but an alternation of generations in the *Rhodophyceæ* will probably not be unqualifiedly accepted by some botanists until the question of the chromosomes is definitely settled, or until the full significance of the reduction is beyond question.

A comparison of the process of fecundation and the immediate subsequent development in certain *Ascomycetes* and *Florideæ* reveals several striking parallels, or, shall we say, homologies. In the first place the female sexual organ in both groups is in all probability homologous. The carpogonium, or oögonium, of the *Florideæ*, with its large receptive part, the trichogyne, may be compared directly with the oögonium of the *Discomycetes*, *e. g.*, *Pyronema*, and, perhaps, with the carpogonium of the lichen *Collema*. The presence or absence of a trichogyne is, moreover, of secondary importance, as this organ is purely an adaptation to peculiar environmental conditions.

All representatives of this type of sexual reproduction agree in that the egg does not, by self-plasmolysis, separate itself as an individual from the oögonium. Whether the gametes be uninucleate or multinucleate is of little importance as viewed from a phylogenetic standpoint.

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Lastly, the development of the gonemoblast filaments in such forms as *Batrachospermum* and *Nemalion* is certainly paralleled in the ascogenous hyphæ of *Erysiphe*, and for the same reason we may look upon the ascogenous hyphæ of *Pyronema* and *Ascobolus* as homologous with the sporogenous filaments of *Dasya* and *Dudresnya*. The ascogenous hyphæ obtain food later in their development from contiguous vegetative cells existing chiefly for that purpose. In this case a cytoplasmic fusion is not necessary for the purpose of nutrition, although it may possibly occur, but in the *Rhodophyceæ*, because of their aquatic habit, the sporogenous filaments must fuse with the brood cells in order to obtain nourishment from them in the most effective way.

This view of phylogenetic relationship is made more probable by the researches of Thaxter on the *Laboulbeniaceæ*, in which certain representatives are shown to be transitional between the *Florideæ* and the *Ascomycetes*. It is certain that the *Ascomycetes* resemble the red algæ more than they do the lower fungi, yet, as we may conclude with Harper, "whether these resemblances are the result of blood relationship or merely due to that similarity in the chemical constitution of the protoplasm of different organisms, which under similar conditions enables it to develop structures nearly related in appearance out of rudiments which may be extremely diverse, is likely to remain a puzzling question."

COLLEMA.

The much discredited doctrine of Stahl ('77) and others concerning sexuality in certain lichens has received fresh confirmation recently by the researches of Baur ('98) and Darbyshire ('99). Although neither cell nor nuclear fusion has been established beyond all doubt, yet the morphological value of the sexual organs can not be very well questioned.

According to Stahl, as is well known, the sexual organs of *Collema microphyllum* occur in large numbers especially upon the illuminated edges of the rapidly growing vegetative lobes of the thallus. The carpogonium arises some little distance beneath the upper surface of the thallus as an ordinary hyphal branch. The lower part, the ascogonium, consists of a row of short cells coiled up somewhat in the form of a corkscrew, which are distinguished from the other hyphal cells by their larger diameter and denser plasmic contents (Fig. 50, A). The number of cells composing the ascogonium, which makes two or three turns, varies considerably, but may often reach twelve. The ascogonium is continued into a straight filament, the trichogyne, which extends to the upper surface of the thallus. The cells of the tricho-

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gyne are smaller in diameter than those of the ascogonium, and their number varies in the species examined from six to twenty-four. A sharp demarkation between trichogyne and ascogonium does not exist.

The end of the trichogyne which projects above the surface of the thallus is generally short and cylindrical or flask-shaped. In rare cases it ends in two short and nearly equal branches. The free surface of this end cell is covered by a viscid substance which facilitates the adherence of the spermatia that escape in large numbers during moist weather from the flask-shaped male organs, the spermagonia.

Baur ('98), who studied *Collema crispum*, confirms Stahl's observations, and gives additional information concerning details of cell structure. The terminal cell of the trichogyne in *Collema crispum*, which projects above the surface of the thallus, is much larger than the other cells of this organ, being longer, somewhat swollen at the middle, and terminating in a point (Fig. 50, B). It is also provided with a viscid coating.

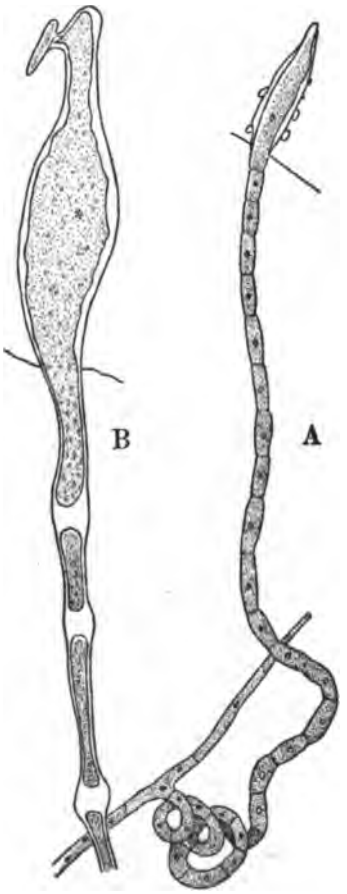

FIG. 50.—Carpogonium of *Collema crispum*. (After Baur.)
A, mature carpogonium; trichogyne ends in large receptive cell which projects above surface of thallus.
B, receptive cell with which a spermatium has fused.

Each cell of the entire carpogonium possesses a nucleus of the typical structure. The transverse walls between the cells are not broken down, though each reveals a small pit, such as is present in the transverse septa of vegetative hyphæ. In four cases Baur found empty spermatia attached to the end of the trichogyne, whose cells showed the same signs of degeneration described by Stahl. The cells in the upper part were collapsed, the cross-walls much swollen, and no nuclei could be seen in them. The septa between the lower cells of the trichogyne were clearly broken down. Each cell of the ascogonium contains at first one nucleus, and since each gives rise to ascogenous

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hyphæ, the pores in the septa may be associated with some part of the process of fecundation. Baur is inclined to regard the first cell of the ascogonium as the egg-cell, attributing to the rest the rôle of auxiliary cells similar to that described by Oltmanns for certain *Florideæ*.

In many cases carpogonia were found which showed no evidence of development into apothecia, their cells giving rise merely to vegetative hyphæ. In these cases no spermatia were found attached to the receptive cells of the trichogyne.

The discovery of a carpogonium in *Physcia pulverulenta* (Schreb.) Nyl. by Stahl and Lindau has been confirmed by Darbyshire. He finds, however, that the cells of the carpogonium become connected by broad strands of protoplasm so as to form almost a single multinucleated cell. Darbyshire shows also the falsity of Lindau's view, namely, that the trichogyne is merely a boring hypha which serves to break a way upward through the thallus for the apothecium.

From the investigations of the authors mentioned there seems to be no doubt that, in the genera in question, the development of the spore fruit is the result of a true sexual process.

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CHAPTER VI.—ARCHEGONIATES.

The preceding chapters have been devoted to the process of fecundation in various typical and well known *Thallophyta*, with the exception of the *Characeæ*, if we may speak of this group as belonging properly to the *Thallophyta*. Owing to the closer resemblance of both sexual organs and gametes to those of certain *Archegoniates*, it has been deemed best to refer to the *Characeæ* in connection with those plants.

Because of our meager knowledge of the development of the spermatozoids, and the union of the sexual nuclei in liverworts and mosses, I have omitted a discussion of the process in these groups and have dealt more fully with sexual reproduction in certain *Pteridophyta* and gymnosperms.

The discovery of spermatozoids in *Cycas* by Ikeno and Hirase, and in *Zamia* by Webber, and a more accurate knowledge of the development of these structures in the *Pteridophyta* have aroused an unusually keen interest in the study of the sexual cells and the phenomena accompanying their union both in these and in the higher plants. In presenting the phenomena relating to the sexual process in the *Archegoniates*, we shall confine ourselves largely to *Onoclea* and *Gymnogramme* among the *Pteridophytes* and to *Cycas*, *Zamia*, *Ginkgo*, and *Pinus* of the gymnosperms; for it is in certain species of these genera that the process, in so far as it has been followed with the use of later methods of research, is best known.

PTERIDOPHYTA.

Until recently the spermatozoid of the *Pteridophyta* was generally conceded by many of the most competent investigators to consist merely of a transformed nucleus with cilia of an obscure cytoplasmic origin. This view was due very largely to the methods of fixing and staining used, which, as we now know, were inadequate to bring out with definite clearness the more delicate cytoplasmic structures of the cell.

In recent years Belajeff, Shaw, and others have applied improved cytological methods to the study of the development of the spermatozoid in *Gymnogramme*, *Onoclea*, *Marsilia* and *Equisetum*. In certain species of these genera, they have found that the mature spermatozoid consists of a nucleus and a delicate band or wing of

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cytoplasm along whose outer edge is a delicate thread or band derived also from the cytoplasm, and from which the cilia are developed (Fig. 52, A). Belajeff was the first to call attention to the cilia-bearing band, which he observed in the development of the spermatozoid in a fern and in *Equisetum*. He also reported a similar body in *Chara*. In speaking of the body which gives rise to the cilia-bearing band, Belajeff used the term "Nebenkern," because of its apparent resemblance to a body of that nature in the spermatid of certain animals. In 1897 Webber described the development of the cilia-bearer in the spermatozoid mother-cell of *Zamia*, and gave to it the name *blepharoplast*.[1] Ikeno and Hirase, who were the first to discover the spermatozoid in certain gymnosperms, described the development of the cilia-bearing band in the spermatozoid of *Cycas* and *Ginkgo*.

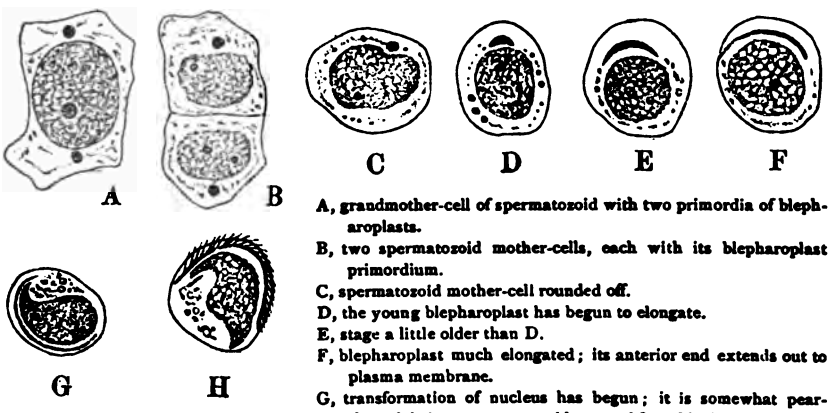

FIG. 51.—Development of spermatozoid in *Gymnogramme sulphurea*.—(After Belajeff.)

A, grandmother-cell of spermatozoid with two primordia of blepharoplasts.
B, two spermatozoid mother-cells, each with its blepharoplast primordium.
C, spermatozoid mother-cell rounded off.
D, the young blepharoplast has begun to elongate.
E, stage a little older than D.
F, blepharoplast much elongated; its anterior end extends out to plasma membrane.
G, transformation of nucleus has begun; it is somewhat pear-shaped, being concave on side turned from blepharoplast; end which will be anterior in mature sperm is pointed.
H, later stage; cilia have been developed from the blepharoplast.

Belajeff and the two Japanese investigators consider the body developing into the blepharoplast as a centrosome. The author is convinced that it has been clearly proved that the blepharoplast is not a centrosome, nor, as yet, has any phylogenetic relationship been shown to exist between the blepharoplast and the centrosome as we know this structure in plants.[2]

THE SPERMATOZOID.

The development of the spermatozoid in *Onoclea*, as described by Shaw ('98), is quite similar to that of *Gymnogramme* according to

[1] From βλεφαρις, eyelash or cilium; and πλαστος, formed.

[2] See Introduction, p. 46.

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Belajeff ('98). Prior to the division of the grandmother-cell of the spermatozoid, *i. e.*, the last cell-division in the spermogenous tissue of the antheridium, which gives rise to the cells that develop directly into the spermatozoids, there appears on opposite sides of the nucleus a small globular body of a homogeneous structure, staining rather densely (Fig. 51, A). These bodies are not provided with any radiations. In *Onoclea* there is, immediately surrounding the nucleus, a region of less granular cytoplasm from which, undoubtedly, the weft of spindle fibers is developed. These bodies, which are the primordia of the blepharoplasts, lie just at the outer edge of this region or weft (Fig. 51, A). In the telophase a blepharoplast primordium lies near the depression of each daughter-nucleus, very near the pole of the spindle (Fig. 51, B, C). Each appears now to be a hollow globular vesicle. Soon after cell-division is completed the development of the daughter-cells directly into spermatozoids begins. The blepharoplast primordium becomes somewhat lens- or crescent-shape in *Gymnogramme*, with the concave side turned toward the nucleus. The nucleus at the same time becomes flattened upon one side and gradually passes into a crescent- or pear-shaped body (Fig. 51, D, E). The blepharoplast has elongated into a thread or band, which follows the convex side of the nucleus and is rather close to it. One end of the band now extends beyond that end of the nucleus which will be anterior in the mature spermatozoid (Fig. 51, F, G). With further development the blepharoplast moves away from the nucleus to a position just beneath the plasma membrane (Fig. 51, H). At this stage the cytoplasm in *Onoclea* (Shaw, '98) shows a depression corresponding to the concave side of the nucleus. At about this period in the development in *Gymnogramme*, according to Belajeff, the cilia make their appearance as outgrowths of the blepharoplast. The nucleus elongates, becoming more slender, and gradually assuming a spiral or corkscrew shape of two or three turns. In the mature spermatozoid (Fig. 52, A) the nucleus is thicker, tapering abruptly, and sometimes to a point, at the posterior end, but gradually forward into a slender anterior end. It is oval in cross section, or, in some cases, slightly flattened on the inner side, especially in the thicker posterior part. In mature spermatozoids of *Onoclea struthiopteris*, fixed and stained on the slide, the cytoplasmic part seems to be in the form of a band which conforms to the spiral course of the nucleus. It is broadest at the anterior end, which extends a short distance, about one or two turns, beyond the anterior end of the nucleus, but it narrows gradually backward, disappearing at a point which marks the thickest part of the nucleus

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(Fig. 52, A). Along the outer edge of the cytoplasmic band extends the blepharoplast as a thread or narrow band from which the cilia arise. The blepharoplast reaches almost or quite to the anterior extremity of the cytoplasmic part, but it cannot be traced farther back than the posterior extremity of the cytoplasmic part, although it may extend some distance farther as a delicate thread closely applied to the nucleus. The blepharoplast is broadest at its anterior end, where it seems to be not perfectly flat, but curved, appearing as a double line, or in cross section as a shallow U. It is, however, very small, so that the exact shape is difficult to determine with certainty. As already stated, it becomes a very delicate thread at the posterior end which is brought

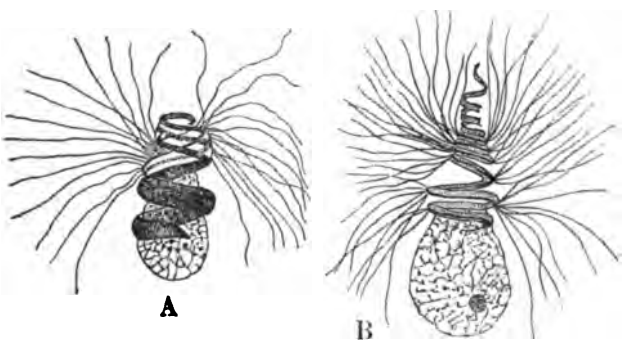

FIG. 52.—Two mature spermatozoids drawn from specimens that were fixed and stained upon the slide a few minutes after their escape from the antheridium.
A, *Onoclea struthiopteris*; B, *Marsilia vestita*.

close to the nucleus by the narrowing of the cytoplasmic band. It is probably for this reason that it cannot be traced after coming into contact with the nucleus. There is nothing to indicate that the blepharoplast extends to the posterior end of the nucleus. The cilia begin at a short distance from the anterior end, and extend backward about two and one-half or three turns. Their length equals or even exceeds that of the spermatozoid when extended.

Judging from Belajeff's figure of a mature spermatozoid, it would seem that the cytoplasm envelops the entire nuclear portion, but in my own preparations, which were made by killing and staining the spermatozoids upon the slide after they had escaped from the antheridium, no cytoplasmic mantle was seen to surround the posterior part of the nucleus. Thom ('99) states also that the whole nucleus is surrounded by a cytoplasmic envelope. It is possible, of course, that the plasma membrane, or even a thin layer of cytoplasm, may envelop the nuclear portion. The nucleus usually appears homogeneous in structure, but

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in some cases in which the stain was well washed out the structure appeared coarsely reticulate or granular. This was observed in spermatozoids of *Onoclea struthiopteris* that were killed on the slide in chrom-osmic-acetic acid and stained in safranin gentian-violet and orange G.

The posterior turns of the spermatozoid embrace the vesicle, which presents a very fine reticulum, and in which coarse granules are held, among them being small starch grains. The author has observed that the vesicle of *Onoclea struthiopteris* became separated from the spermatozoids a short time after their escape from the antheridium; for, of the many hundreds fixed and stained upon the slide a few minutes after their escape from the antheridia, relatively few were found with the vesicle adhering.

The development of the spermatozoid of *Marsilia*, according to Shaw ('98) and Belajeff ('99), differs in certain important details from that of *Onoclea*. As this process is known in so few of the *Pteridophyta*, it is perhaps well to present briefly the facts as they are known in one of the heterosporous forms.

At the close of the second from the last division in the spermogenous tissue of *Marsilia vestita*, or that leading to the great-grandmother-cell of the spermatozoid (the primary spermatocyte of Shaw), there appears at each pole of the spindle, or near it close to the daughter-nucleus, a small body which is called by Shaw a *blepharoplastoid*. During the resting stage of the nucleus the blepharoplastoid seems to divide. The two halves increase in size and remain together near the nucleus. As soon as the nucleus of the great-grandmother-cell begins to divide, the pair of blepharoplastoids move away from the nucleus and remain at a position in the cytoplasm between one pole of the spindle and the equatorial plane, until the metaphase, or early anaphase, when they disappear. About the same time, or a little later, a small blepharoplast appears near each pole of the spindle. At the close of the division the blepharoplast lies near the nucleus of the grandmother-cell of the spermatozoid (secondary spermatocyte or spermatocyte mother-cell of Shaw). It now divides, and the two daughter blepharoplasts increase in size and separate from each other, at the same time moving away from the nucleus (Fig. 53, A, B). Each takes a position near the pole of the future spindle but always a little to one side of its longitudinal axis. They increase in size and remain apparently unchanged in structure until the anaphase, when each seems to be hollow (Fig. 53, B, C).

As soon as the nucleus of the spermatozoid mother-cell (spermatid)

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is formed, a small eccentric body appears in each blepharoplast (Fig. 53, D), then several, so that it appears as if the blepharoplast had broken up into a group of small bodies (Fig. 54, E). Out of these bodies is developed the band, which elongates, and together with the nucleus moves toward the plasma membrane of the cell (Fig. 54, F, G). In cross section the band is broadly U-shaped, but when seen from above it appears as a double line (Fig. 54, H). The band continues to elongate until finally a spiral is formed, which makes five or more turns about the hemispherical half of the cell (Fig. 54, I). The nucleus also elongates, becoming sausage-shaped, and lies in close contact with the larger turns of the blepharoplast. The mature spermatozoid in *Marsilia* is composed, therefore, of a blepharoplast,

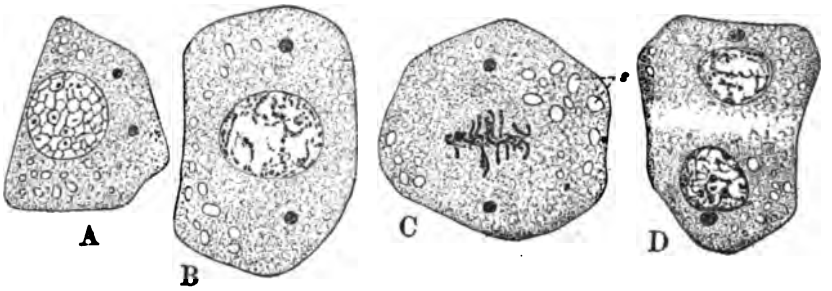

FIG. 53.—Blepharoplast primordium during division of grandmother-cell of spermatozoid in *Marsilia vestita*.—(After Shaw.)

A, the two primordia of the blepharoplasts lie in cytoplasm some distance from nucleus.

B, they are now on opposite sides of the nucleus but a little to one side of median line.

C, the nucleus is in spindle stage of division; the young blepharoplasts lie near the respective poles of spindle.

D, telophase of division; blepharoplast rudiment at pole of each nucleus contains a dense granule.

consisting of a funnel-shaped spiral of about ten or more turns, and a sausage-shaped nucleus without a definite visible structure, which is connected with the three larger posterior turns of the blepharoplast (Fig. 52, B). The posterior end of the blepharoplast, which is usually bent in the shape of a hook, extends beyond the nucleus. The relatively large vesicle is embraced by the larger posterior turns of the blepharoplast. In *Marsilia vestita* the author observed that the vesicle remains adhering to the spermatozoid for a longer time than in *Onoclea struthiopteris*. The vesicle consists of a delicate cytoplasmic reticulum, in which are held large starch and protein granules. The numerous cilia (the spermatozoids were fixed and stained upon the slide) spring from the middle and posterior coils, the two or three anterior coils being free from them. In some cases observed the cilia extended almost to the posterior end of the blepharoplast. As soon as the vesicle drops off, the spermatozoid becomes much elongated, losing its pronounced funnel-shape.

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Belajeff ('99), who has also studied the development of the spermatozoid in *Marsilia*, agrees with Shaw in so far as the transformation of the primordium of the blepharoplast into the mature cilia-bearing organ is concerned, but, as regards the earlier behavior of the primordia, these observers disagree in certain important particulars. Belajeff, who regards the blepharoplast as a centrosome, finds that in the division of the grandmother-cell of the spermatozoid, the primordia, which lie some distance from the nucleus, divide, and a faint central spindle is formed between the daughter primordia. This structure, he maintains, gives rise to the karyokinetic spindle just as in some animal

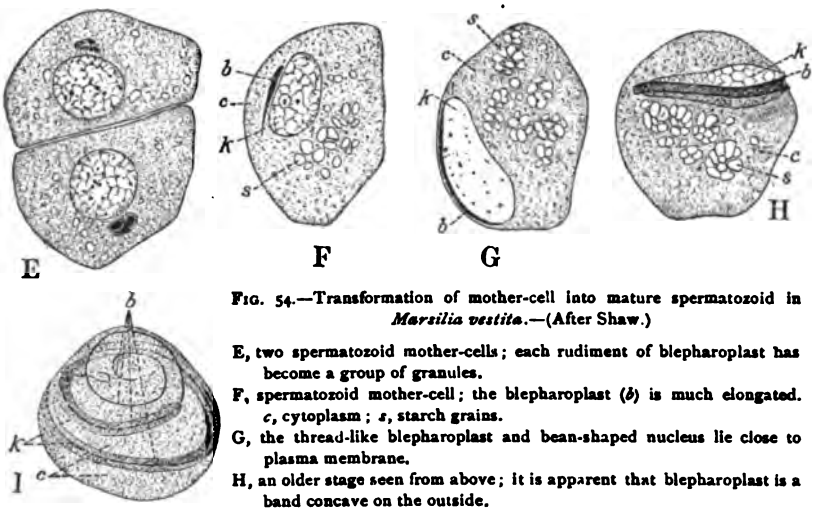

FIG. 54.—Transformation of mother-cell into mature spermatozoid in *Marsilia vestita*.—(After Shaw.)

E, two spermatozoid mother-cells; each rudiment of blepharoplast has become a group of granules.

F, spermatozoid mother-cell; the blepharoplast (*b*) is much elongated. *c*, cytoplasm; *s*, starch grains.

G, the thread-like blepharoplast and bean-shaped nucleus lie close to plasma membrane.

H, an older stage seen from above; it is apparent that blepharoplast is a band concave on the outside.

I, the blepharoplast and sausage-shaped nucleus (*k*) make several spiral turns within the cell close to plasma membrane.

cells, and concludes, therefore, that the blepharoplast primordia are centrosomes. The author has already dealt with this matter in the introductory chapter, and a further discussion will not be given here.

In *Equisetum* Belajeff has found that the spermatozoid develops in a manner similar to that of the fern, and there are good reasons for believing that the process of development is much the same in the majority of archegoniates, although our knowledge is yet too meager to warrant any sweeping generalization.

It seems fitting in this connection to compare the mature spermatozoid of the *Characeæ* with that of the fern. Belajeff ('94) has shown that in the development of the spermatozoid of *Chara fœtida* the two cilia are borne by a thread-like body which arises in the cytoplasm in a manner similar to the blepharoplast of the fern. The spermatozoid,

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as in the *Pteridophyta* and *gymnosperms*, is a transformation of the entire contents of the cell, and we may with much propriety regard the spermatozoid of *Chara* and that of the fern as homologous structures. But whether we are dealing with real homologies, or only with striking analogies, is certainly a question concerning which there may be some diversity of opinion.

The fate of the spermatozoid of *Chara* after penetrating the egg and the union of the two sexual nuclei is practically unknown in detail, and a further discussion of the process of fecundation in the absence of more facts would seem without value, since it is not the purpose to enter here into any discussion of the homologies of the sexual organs of the Characeæ with those of the Archegoniates.

THE EGG-CELL AND FECUNDATION.

In more recent years the process of fecundation has been observed in various genera of the *Felicineæ* by Campbell, in *Onoclea* by Shaw, and in *Adiantum* and *Aspidium* by Thom. The author has followed the process in *Onoclea struthiopteris*, and his observations confirm those of Shaw, who has traced the behavior of the sexual nuclei in great detail in *Onoclea sensibilis*.

Soon after the division which cuts off the ventral canal-cell, and before the archegonium of *Onoclea struthiopteris* is full grown, the three central cells contain fine-meshed and densely granular cytoplasm. Their nuclei are in the resting stage. The wall between egg and ventral canal-cell is generally arched slightly downward into the egg-cell. This wall is laid down in this position, at least in many cases, and the concave upper surface of the egg does not seem to be due to pressure from the ventral or neck canal-cell.

As the archegonium matures it increases in size, and the cytoplasm of the central cells becomes looser. A rather large vacuole has been observed in the ventral canal-cell in the mature organ. It is well known that in *Onoclea* the nucleus of the neck canal-cell often divides, but a division of the cell does not follow, except, possibly, in rare cases. The daughter-nuclei are reconstructed and lie usually close to each other. The author has observed in several instances that the division of the neck-canal nucleus took place at exactly the same time as the division of the central cell which cuts off the ventral canal-cell. Whether any special significance should be attached to this phenomenon the author is unable to state. Observers have often been tempted to consider the ventral canal-cell as a rudimentary egg, but if there be good grounds for such a view it is, perhaps, as much in harmony with the facts to regard the neck canal-cell or cells as aborted eggs.

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The entrance of the living spermatozoid into the neck of the archegonium and its passage down to the egg is easily followed. In fact, the phenomenon is a matter of common observation in elementary classes. It is only necessary to mount prothallia with mature archegonia ventral side up in a drop of water, to which are added several clean male prothallia that contain ripe antheridia, and which have been kept in dry air for a short time previous to the operation. The ripe archegonia will open, and in a few minutes numerous spermatozoids which have escaped on being placed in the water will be found swimming about the opening of the archegonium, having been attracted thither by the extruded substance. Many enter the neck, and several may reach the egg-cell. The author has observed instances in which the number of spermatozoids endeavoring to enter the archegonium was so great that they formed a plug which almost completely closed the opening in the neck.

Since the interesting researches of Pfeffer ('84) it has been known that the mucilaginous substance formed from the neck-canal and ventral-canal cells acts as a chemotactic stimulus upon the spermatozoids. Pfeffer found that the spermatozoids of ferns are attracted by malic acid and its salts in very dilute solutions. A solution of 0.001 grm. per cent. is sufficient to bring about a positive chemotactic reaction.

Buller (1900) found that in addition to malic acid and its salts, many organic and inorganic salts, widely occurring in the cells of plants, exercise a positive chemotactic stimulus upon the spermatozoids of certain ferns. Among the organic salts which were found to attract are tartrates, potassium oxalate, potassium acetate and sodium formate. Among the inorganic salts are phosphates, sulphates, potassium nitrate and potassium chloride. Organic substances which were found to act indifferently are grape sugar, cane sugar, lactose, amylodextrine, glycerine, alcohol, asparagin and urea. "Inorganic salts not appreciably attracting are the chlorides and nitrates of sodium, ammonium and calcium, and also lithium nitrate. Of the four free acids which seem to be most widely found in cell-sap, namely, malic, oxalic, tartaric and citric, only malic acid attracts." The concentration of malic acid which gives the most pronounced reaction is 0.01 grm. per cent., while that which gave just an appreciable reaction was 0.001 grm. per cent. With potassium nitrate no attraction could be detected at 0.05 grm. per cent., whereas there was a slight one at 0.1 per cent. Roughly estimated, therefore, malic acid attracts fifty times more strongly than potassium nitrate. Strong solutions repel.

Attempts have been made to elucidate the phenomena of chemotaxis

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by means of the theory of electrolytic dissociation of solutions, and with some success. As regards the spermatozoids of ferns, Buller has shown that in the case of some compounds, as certain salts of potassium and malic acid, the attraction is probably due to certain ions. It is not to be assumed, however, that a chemotactic stimulus may be given only by ions, for certain substances which are not dissociated have been found to exert a chemotactic stimulus. In this connection it is interesting to note that Pfeffer found that the spermatozoids of mosses are attracted by cane sugar, which does not attract the spermatozoids of ferns.

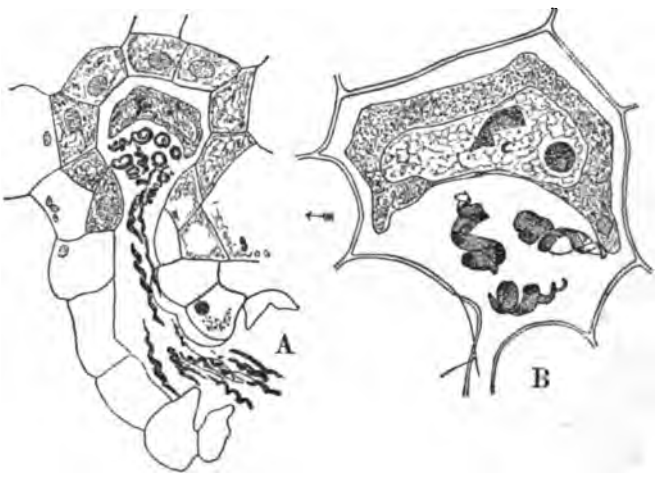

FIG. 55.—Archegonium of *Onoclea sensibilis*.—(After Shaw.)

A, vertical section through an open archegonium, probably within ten minutes after entrance of first spermatozoid; an unchanged spermatozoid is inside egg-nucleus.

B, vertical section of venter of an archegonium containing spermatozoids, and a collapsed egg with a spermatozoid within nucleus: thirty minutes.

Although malic acid exerts a strong chemotactic stimulus upon the spermatozoids of certain ferns, yet from the foregoing it is evident that the attraction by the mucilaginous substance extruded from the archegonium is not, of course, a decisive proof that malic acid compounds are present in that substance.

Before the archegonium opens the egg-cell is concave on the upper side. The nucleus is also flattened or concave; it is in the resting stage and may contain one or more nucleoli. Shaw has observed that, in living sections, the egg swells as soon as the canal is cleared of its dissolving contents, and fills the venter. That part which was previously concave now forms the receptive spot. In fixed and stained preparations the author has found this same condition of the egg-cell

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when the neck-canal contained many spermatozoids, and when one lay against the receptive spot, but had not penetrated.

On entering the extruded mucilaginous substance the spermatozoids leave their vesicles behind, and their motion is retarded. The cork-screw spiral is drawn out and the number of turns apparently increased. The forward motion of the spermatozoid is accompanied by a rotation which corresponds to the pitch of the screw.

The behavior of the spermatozoid after entering the egg can be followed only in properly fixed and carefully stained sections. Shaw found that in all prothallia killed within an hour after the entrance of the spermatozoid into the archegonium the egg-cells were in a collapsed condition, being concave on the outside, and the nucleus conforming to the shape of the cell (Fig. 55, A). The concavity of the egg-cell occupies the position of the receptive spot. This condition was regarded by Shaw as normal, and not the result of killing reagents, since in the living condition spermatozoids were seen moving freely in the cavity above the egg. I quote as follows:

> There are reasons to believe, however, that the collapse is not an artificial plasmolysis, but that it takes place as soon as the spermatozoid enters the egg. The mature egg has been described (for the other species, *O. struthiopteris* (Campbell, '95)) as having a large hyaline receptive spot. The concavity of the collapsed egg occupies the position of that spot. That it was formed before the plants were killed seems evident from the movement of a number of spermatozoids in the venter. This can be seen in the living plants. That the number of these spermatozoids is large is shown by the specimens stained and sectioned. They could hardly have been carried into the venter by the fixing agent, for those in the canal were fixed first, in the extended condition, and those in the venter afterward in the contracted form. From the evidence at hand it appears that as soon as the egg is entered by a spermatozoid it loses its turgidity, and the spermatozoids which come into the venter afterward meet with little or no resistance from the egg. It may be that the turgid condition of the egg, in the first place, offers mechanical facility for the screw-like spermatozoid coming through the narrow base of the neck to force itself into the cytoplasm of the receptive spot, and that the plasmolytic condition of the egg afterward deprives the following spermatozoids of this advantage, and protects the egg from injury or from multiple fertilization by them.

In sections made from material killed in both chrom-acetic and chrom-osmic-acetic acid the author has also observed in many cases the collapsed condition of the egg-cell as described by Shaw. Several preparations were, however, especially interesting as they tend to throw some doubt upon the collapsed condition being a normal occurrence. In one of these two or more spermatozoids had entered the egg, one of which, or rather its nucleus, had partly penetrated the egg-nucleus;

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the others lay in the cytoplasm of the receptive spot (Fig. 56, C). (In this figure one of the spermatozoids was cut in sectioning, so that only two separate pieces of it are shown, the other parts being in the next section.) The nucleus was concave above, but the egg-cell had not collapsed. It remained apparently turgid, having been only slightly shrunken uniformly on all sides by the reagents. The membrane of the egg seemed to be firm, but whether it was anything more than a plasma membrane I was unable to determine. The prothallium from which this preparation was made was killed in chrom-acetic

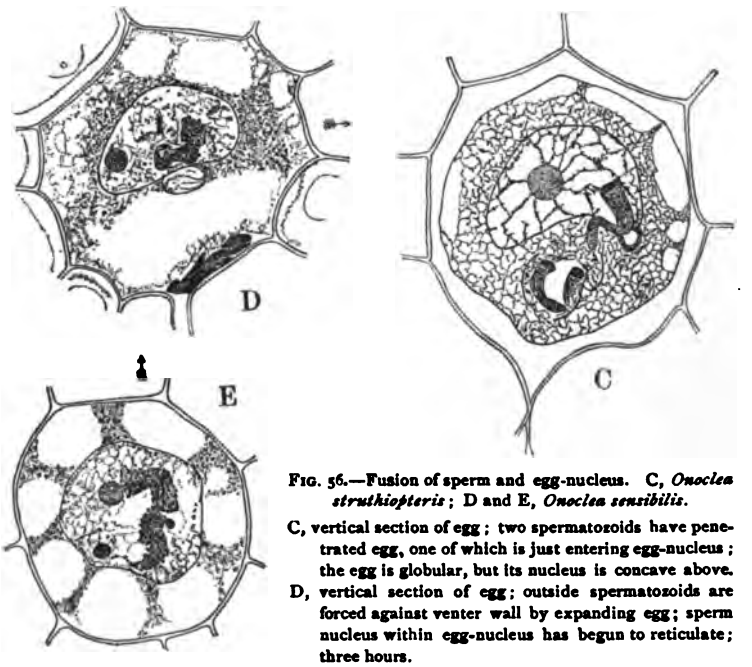

FIG. 56.—Fusion of sperm and egg-nucleus. C, *Onoclea struthiopteris*; D and E, *Onoclea sensibilis*.

C, vertical section of egg; two spermatozoids have penetrated egg, one of which is just entering egg-nucleus; the egg is globular, but its nucleus is concave above.

D, vertical section of egg; outside spermatozoids are forced against venter wall by expanding egg; sperm nucleus within egg-nucleus has begun to reticulate; three hours.

E, horizontal sectional section of an egg; fourteen hours. (D and E, after Shaw.)

acid, and, although stained on the slide with Bismarck brown in addition to the Flemming triple stain, there was nothing to indicate with any certainty a cellulose character of the membrane. Lying in the cytoplasm near the nucleus of each spermatozoid was a delicate thread which seemed to be the blepharoplast. The cytoplasmic reticulum was somewhat shrunken from the membrane of the egg on one side. In another preparation mentioned in a preceding paragraph the opening of the neck of the archegonium was apparently closed by a plug of spermatozoids after one had entered. This spermatozoid lay against

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the oval surface of the receptive spot, but had not penetrated the plasma membrane. It had apparently untwisted and had begun to reticulate, as its structure was somewhat granular or lumpy in appearance. In still another instance the spermatozoid had just passed through the plasma membrane at the receptive spot. The egg was not collapsed, but quite turgid. The receptive spot was distinguished from the rest of the cytoplasm only by the presence of fewer granules and, perhaps, a little looser reticulum. Other eggs were observed in a turgid condition (the archegonium being open), into which no spermatozoid had penetrated, but the nucleus was concave on the upper side. It may be mentioned that the nucleus is not always concave, but may be rounded or globular. Apart from these instances the observations of the author agree with those of Shaw.

In about one-half hour, or less, after the entrance of the spermatozoid into the archegonium, the canal is closed by the expansion of the four proximal neck-cells and the four just beyond them. The egg recovers its turgidity and forces the free spermatozoids against the outer wall of the venter (Fig. 56, D). A cellulose membrane does not seem to be formed about the egg immediately, although, as stated by Shaw, a very delicate cellulose wall may have been dissolved by the chromic acid used in fixing. Soon after penetrating the egg the nucleus of the spermatozoid enters the egg-nucleus before undergoing any change in form or visible structure (Fig. 55, B). The fate of the cytoplasmic part was not very satisfactorily followed, but all the facts observed indicate that the cytoplasmic band and blepharoplast are left in the cytoplasm of the egg, where, as in *Cycas* and *Zamia* of the Gymnosperms, they are absorbed. In Fig. 56, D, a body lying near the concave side of the nucleus bears some resemblance to the cytoplasmic part of the spermatozoid. The author has also observed in several instances undoubted traces of the blepharoplast near the upper surface of the nucleus, and there is no question but that the fate of the blepharoplast and cytoplasm is as just stated.

The egg-nucleus during the entire process of fecundation is in the resting condition. Several conspicuous nucleoli are usually present. They vary in size and have a vacuolate structure. In the delicate linin network are distributed the small chromatin granules.

In a short time the sperm-nucleus within the egg-nucleus begins to reticulate, becoming visibly granular and of a looser structure. This is apparent three hours after the entrance of the spermatozoid into the archegonium (Fig. 56, D), but it may sometimes be seen earlier, after thirty minutes or one hour. The time after which a change is notice-

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able in the sperm-nucleus varies greatly in different individuals. In some cases the sperm-nucleus, after two days, showed no further advance than was observed in others after only thirty-six hours. As the reticulation of the sperm-nucleus continues, its structure becomes looser and more open, and its cork-screw shape disappears (Fig. 56, D, E). As far as is known at present the reticulation of the sperm-nucleus continues until its network is no longer recognizable from that of the egg when fecundation is complete.

During the process of fusion it will be seen that the sperm-nucleus goes through the same series of changes as in the development of the spermatozoid, but in the reverse order. The time elapsing between the entrance of the sperm-nucleus into the egg and complete fusion may vary considerably in individual cases.

In *Pilularia globulifera*, according to Campbell ('88), the sperm-nucleus assumes a loose and more granular structure, and rounds up before penetrating or uniting with the nucleus of the egg. Judging from Campbell's figures, it seems that in *Osmunda* (Campbell, '92) the sperm-nucleus, as in *Onoclea*, enters the nucleus of the egg before undergoing any visible change in form or structure.

In this respect certain ferns are without parallel in the plant kingdom, except, perhaps, in the Gymnosperms, and it would be interesting to know how widely distributed the phenomenon is in the *Pteridophyta*, and whether it occurs in any other plants.

GYMNOSPERMS.

CYCAS, ZAMIA, AND GINKGO.

THE MALE GAMETOPHYTE.

The development of the spermatozoid in *Cycas* (Ikeno, '96, '98), *Ginkgo* (Hirase, '96, '98; Webber, '97; Fujii, 1900), and Zamia (Webber, '97, 1901), bears a striking resemblance to that in the fern, especially in regard to the origin and behavior of the blepharoplast. There seems now to be no doubt that the blepharoplast in these three genera is homologous to the blepharoplast of the fern, and, in fact, the entire development of both sexual cells indicates with a certainty that these gymnosperms bear a close phylogenetic relationship to the pteridophytes.

Since the development of the spermatozoid in *Cycas* and *Zamia* differs in certain important details according to the two investigators, Ikeno and Webber, a somewhat detailed account of the process will be given for both genera, while *Ginkgo* will be referred to for comparison.

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The mature microspore of *Cycas revoluta*, according to Ikeno, consists of a large tube cell the so-called vegetative cell, which gives rise to the pollen tube, and two smaller prothallial cells (Fig. 57, A, p_1, p_2). The nucleus of the tube-cell is large, and contains a loose thread-work and a nucleolus. The nuclei of the prothallial cells are smaller, and flattened to conform with the shape of those cells. The

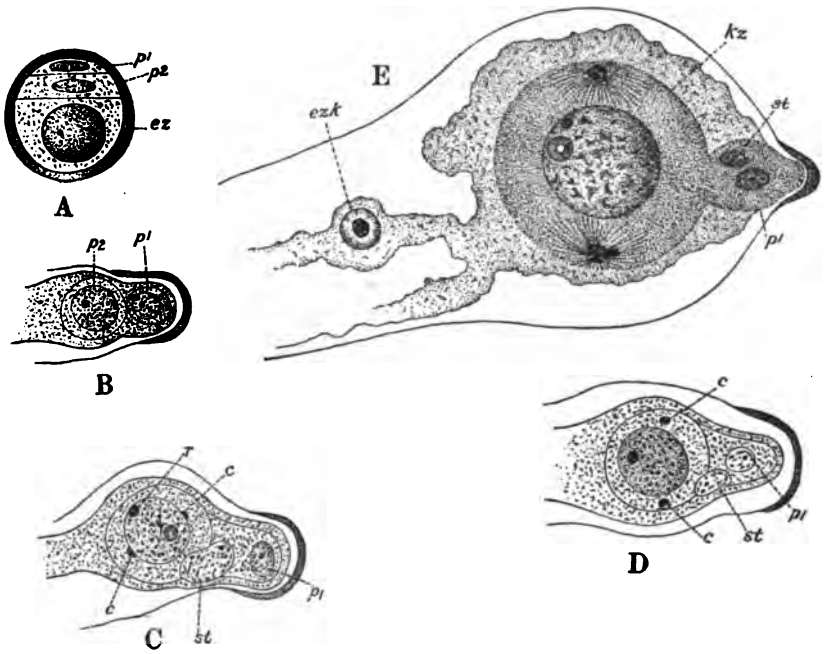

FIG. 57.—Microspore and development of male gametophyte in *Cycas revoluta*.—(After Ikeno.)

A, mature microspore. p_1, outer, p_2, inner prothallial cells; *ez*, tube cell.

B, proximal end of pollen tube capped by exine of spore; two prothallial cells, p_1 and p_2, have rounded off and increased in size.

C, same at later stage of development; the inner prothallial, or antheridial, cell has divided into the generative cell and stalk cell (*st*); p_1, first prothallial cell; *c*, *c*, primordia of blepharoplasts; *r*, nucleolus of generative cell nucleus.

D, later than C; the blepharoplast primordia (*c*) have moved away from nucleus.

E, proximal end of pollen tube shortly before division of generative cell (*ks*) which has increased greatly in size; the large blepharoplasts are provided with beautiful radiations; the tube nucleus (*ezk*) has migrated back into proximal end of tube.

walls cutting off the prothallial cells, according to Ikeno, are straight, meeting the wall of the pollen spore, while in *Zamia* Webber finds that these walls, which are only plasma membranes, are arched out into the tube cell. The inner cell (p_2) gives rise to the antheridium, and may be known as the antheridial cell.

A period of about three months elapses between pollination, which takes place early in July, and fecundation in October. Immediately

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after pollination each spore in the pollen chamber of the macrosporangium germinates, the tube cell developing gradually into a branched tube which penetrates the tissue of the nucellus. The tube-nucleus passes into the tube, maintaining a position near the growing region or end as long as the tube continues its growth into the tissue of the nucellus, while the two prothallial cells retain their former position. Contrary to the genus *Pinus* and other higher *Conifers* the distal end of the tube does not grow directly toward the archegonia, but laterally and downward, serving especially as an organ for the absorption of food (Fig. 65, A). The proximal end of the tube, carrying before it the cap of exine, or the remaining outer wall of the spore, finally grows toward the archegonium. The pollen tube has a similar behavior in *Zamia* (Webber, '97) and *Ginkgo* (Hirase, '98).

Soon after the germination of the spore the two prothallial cells increase in size, especially the antheridial cell, which becomes spherical (Fig. 57, B, p_2). Its nucleus is also correspondingly large, and the cytoplasm presents a looser structure. In the meantime the antheridial cell divides, the daughter-nuclei being of equal size. According to Ikeno ('98, p. 172) a wall is not formed between these two nuclei in *Cycas revoluta*. One of them now increases rapidly in size, so that it occupies nearly the entire cavity of the mother-cell, while the other remains small and is crowded out as a naked nucleus (Fig. 57, C, D, *st*). The larger cell is known as the *generative cell* (Körperzelle of the German literature) and gives rise to two spermatozoids; the smaller cell is the *stalk cell* (Fig. 57, C, D, *st*).

As we shall see later Webber finds that the antheridial cell divides regularly into the stalk and generative cells, but the plasma membrane separating the two cells is delicate, and the stalk cell arches over the first prothallial cell in such a manner as to give the appearance of the latter being nearly enclosed by the former (Fig. 60, F, G). It is possible that the same is true also for *Cycas*. The plasma membrane, being very delicate, may have been overlooked by Ikeno, for the position of the two cells is such as to make it appear that the stalk nucleus was forced out of the mother-cell.

Soon after this stage of development two small bodies appear in the generative cell (body-cell), lying close to the nucleus and on opposite sides (Fig. 57, C, *c*). Ikeno seems to be of the opinion that the two bodies, which he calls centrosomes, are derived from the nucleus, for the reason that just prior to their appearance outside of the nucleus, objects staining similarly appear within the nucleus. These bodies, which are the primordia of the blepharoplasts, move away from the

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nucleus toward the periphery of the cell (Fig. 57, D, *c*). With further growth the generative cell with its nucleus becomes elliptical, their major axis lying parallel with the longitudinal axis of the tube. The two primordia of the blepharoplasts, which lay previously in line parallel with the transverse axis of the tube, are now found in the ends of the generative cell. About each there soon appear beautiful kinoplasmic radiations, giving them a most striking resemblance to centrospheres with large centrosomes. Later in the period of development, or about the middle of August in Japan, the young blepharoplasts shift their position again, so that their earlier orientation in the generative cell with respect to the axis of the pollen tube is resumed (Fig. 57, E). The generative cell becomes spherical, and the kinoplasmic radiations are very conspicuous.

From this time until the end of September, or about one and one-half months, few changes manifest themselves in the generative cell apart from an increase in size. This period in the development is, therefore, a period of growth, which corresponds to a similar period in the development of the archegonium, and at the end of which all elements have reached their maximum size (Fig. 57, E). The diameter of the generative cell, which contains dense cytoplasm, is about 0.14 mm., and that of the nucleus is about 60 μ. The primordia of the blepharoplasts have also increased considerably in size; they are about 15 μ in diameter. Apart from the presence of one or more vacuoles, they are rather homogeneous massive bodies. The kinoplasmic radiations are still beautifully developed; they seem to pass over gradually and insensibly into the alveolar structure of the cytoplasm.

About the middle of September the tube nucleus begins to migrate toward the proximal end of the pollen tube, and, by the end of the month, this nucleus, the generative, stalk, and outer prothallial cells are all in the proximal end, which is capped by the exine of the spore. It may be mentioned here that the migration of the tube nucleus into the proximal end of the pollen tube seems to be a striking confirmation of the doctrine of Haberlandt, namely, that in a growing cell the nucleus generally takes a position near the seat of constructive activity. Since the proximal end of the tube now grows toward the archegonium, and as growth at the distal end ceases, it is to be expected, in harmony with the theory of Haberlandt, that the nucleus which presides over this growth should move toward the region of that activity. Webber has observed the same behavior of the tube nucleus in *Zamia*.

The final processes which now take place in the male gametophyte have to do largely with the development of the two spermatozoids

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from the generative cell. To this phase of development Ikeno has applied the term spermatogenesis.

As soon as all the structures mentioned accumulate in the proximal end of the tube, all save the generative cell begin to disorganize and finally disappear. What this disorganization signifies, Ikeno remarks,

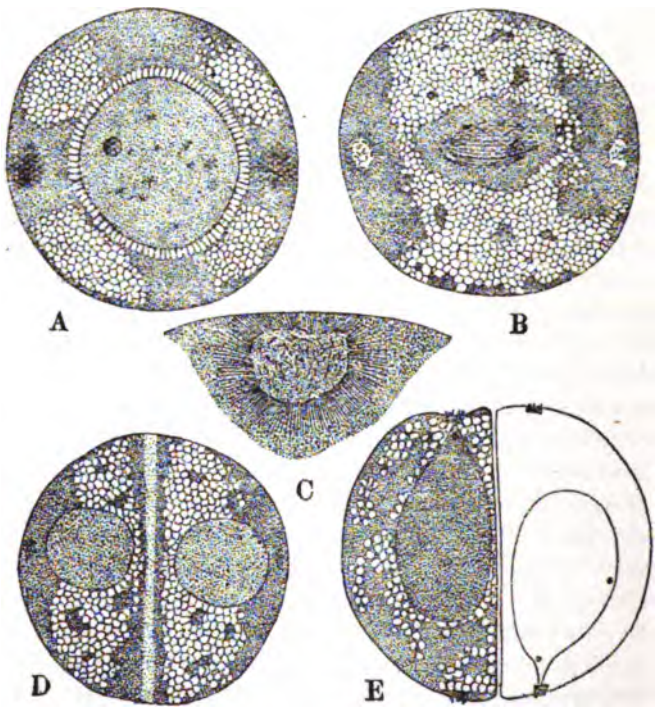

FIG. 58.—Division of generative cell and further development of blepharoplasts in *Cycas revoluta*.—(After Ikeno.)

A, generative cell with nucleus in early prophase of division; chromatin scattered in masses of granules.
B, same with nucleus in late anaphase; each blepharoplast has separated into a mass of rods from which radiations extend; they have nothing whatever to do with mitotic spindle.
C, blepharoplast of B more highly magnified.
D, cell-division is about complete; the radiations have nearly disappeared from the mass of granules composing blepharoplast.
E, two spermatozoid mother-cells, the one on the right in outline; the ciliated blepharoplast has made one turn about the cell; nuclear beak is in connection with ciliated band.

is an open question, but it seems that all of the disorganized elements contribute to the nourishment of the generative cell.

The cytoplasm of the generative cell now assumes a coarse, net-like structure, and the nucleus divides (Fig. 58, A, B). The details of this division will not be dwelt upon further than to state that the mitotic spindle arises without the intervention of the centrosphere-like

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primordia of the blepharoplasts (Fig. 58, B). This is true for *Zamia*, according to Webber, and for *Ginkgo*, according to Hirase. At this stage each primordium of the blepharoplast is transformed into a group of fine rods about which the radiations, although not so pronounced, are still present (Fig. 58, C). When, however, the daughter chromosomes have arrived at the poles of the spindle, each blepharoplast has become a mass, or an accumulation, of granules, and the radiations can scarcely be recognized.

At the close of nuclear division each daughter-nucleus is homogeneous, presenting a small number of nucleoli. A cell-plate is formed and the division of the generative cell completed (Fig. 58, D). The next step is characterized by the behavior of the mass of granules of the young blepharoplast. These are arranged close to the nucleus into a more or less short and broad band whose granular nature is still evident. Seen in profile a number of radiations appear extending out from the band toward the periphery of the cell (Fig. 59, A). These radiations are the developing cilia of the spermatozoid. Whether the cilia are transformed radiations, or arise anew, is a question. Ikeno ('98, p. 180) is inclined to think that the former mode of origin is the more probable.

In the meanwhile the nucleus develops a beak which becomes connected with the ciliated band (Fig. 59, A). The development of the nuclear beak and the arrangement of the granules into a band take place simultaneously, so that it is not known which phenomenon is of first importance. If the formation of the beak took the initiative, then it would be reasonable to suppose that the direct coöperation of the nucleus in the development of the band is indispensable. In *Zamia*, according to Webber, no such nuclear beak occurs in the development of the spermatozoid. Subsequent to this stage in the development of the band its granular nature is no longer recognizable; it appears as a thin homogeneous thread (Fig. 59, B). The further behavior of the blepharoplast seems to be characteristic of spermatogenesis in *Cycas*, *Zamia*, and *Ginkgo*. The ciliated band extends itself in a spiral which ultimately makes five turns around the hemispherical cell, always remaining near its surface just beneath the plasma membrane. During this process the nucleus increases in size and becomes somewhat pear-shaped. Its beak, to which is attached apparently one end of the band, increases in length until it almost reaches the surface of the cell (Fig. 58, E, and Fig. 59, B). The free end of the band continues its spiral course around the cell a short distance beneath the plasma membrane. The direction of the spiral is parallel with the

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plane of division of the generative cell. In Fig. 58, E, which represents a median section through the two daughter-cells, the blepharoplast has made one turn around the cell. The cilia, which at first lay wholly within the cytoplasm, project out through the plasma membrane as the band approaches the surface of the cell. The nuclear beak, which remains in close contact with the band during its earlier development, finally becomes separated from it (Fig. 59, C). In the mature spermatozoid the blepharoplast, as already stated, makes about five turns around the cell counter clock-wise. As is evident from a median

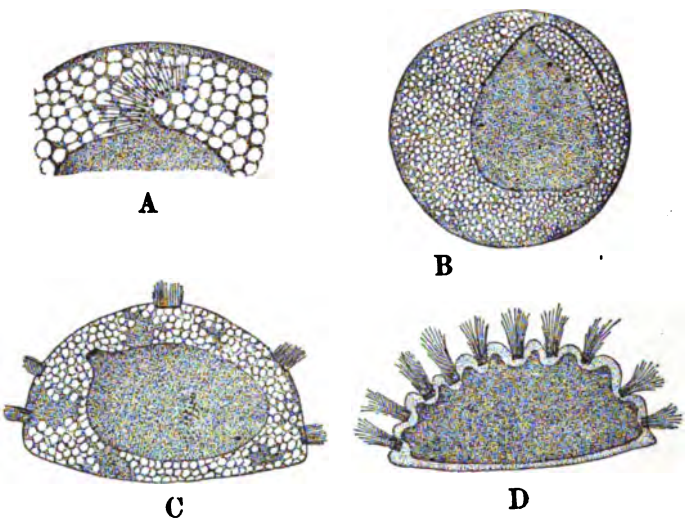

FIG. 59.—Further development of spermatozoid in *Cycas revoluta*.—(After Ikeno.)

A, part of spermatozoid mother-cell showing nuclear beak in contact with granular blepharoplast band.
B, spermatozoid mother-cell; band-shaped blepharoplast longer, one end being applied to nuclear beak.
C, later stage; blepharoplast has made about three turns about the cell; the nuclear beak seems to have separated from ciliated band.
D, nearly ripe spermatozoid in median section. Both nucleus and cytoplasm are lobed on one side as if constricted by blepharoplast, which describes about five turns around the hemispherical cell.

section, the mature spermatozoid consists of a large nucleus completely surrounded by a thin layer of cytoplasm, and the blepharoplast lies in a depression or groove (Fig. 59, D). As a result both cytoplasm and nucleus are lobed, thus presenting a wavy contour in section. This phenomenon seems to indicate that during the final increase in size of the nucleus, the blepharoplast acted as a kind of constriction upon the anterior end of the cell. The same is true in both *Zamia* and *Ginkgo*. In the mature spermatozoid the cytoplasm which completely surrounds the nucleus is clearly distinguishable. As will be seen for *Zamia* and

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Ginkgo, the spermatozoid of *Cycas*, as has been pointed out for the fern, is a transformation of the entire mother-cell.

The development of the spermatozoid in both *Ginkgo* and *Zamia* closely resembles that in *Cycas*. That in *Zamia* differs, however, according to Webber, in certain important details, and because of this fact the process in *Zamia* will be given also in some detail. Webber investigated two species growing in Florida—*Zamia floridiana* and *Z. pumila*.

As a rule the mature microspore of *Zamia* consists of the tube cell and two prothallial cells (Fig. 60, A). Only in exceptional cases were evidences of a third cell observed, but if three prothallial cells are formed in the development of the pollen spore as is claimed for *Cycas*, the first is generally absorbed before the spore is mature, leaving only a trace in the form of a dark line. The two prothallial cells are provided with only a plasma membrane. The first prothallial cell is shaped like a plano-convex lens and arches out into the second prothallial cell. The second prothallial cell is attached to the first and arches out into the tube cell (Fig. 60, A, B). This is especially marked during the growth of the pollen tube. The nucleus of the tube cell is larger than those of the prothallial cells, and of the latter the nucleus of the first is larger than that of the second. Very soon in the growth of the pollen tube the second or antheridial cell, together with its nucleus, greatly exceeds the first.

The process of pollination, which occurs in Florida in January, brings the pollen grains into the pollen chamber, a cavity in the apex of the nucellus, formed by the disorganization of the tissue of the latter. Webber ('01) states that the passage of the pollen grain through the micropyle is evidently accomplished by suction.

A somewhat mucilaginous fluid is secreted by the cells which surround the micropyle, and a drop of this fluid is probably protruded at the time of pollination. The fluid disappears later, and during the formation of the pollen chamber a suction is formed by the breaking down of the cells in its formation, so that the fluid, together with the pollen grains that may be held in it, is brought down into the pollen chamber.

In a short time after the pollen grains have been brought into the pollen chamber they germinate, the tube bursting out of the exine of the grain at a point opposite the prothallial cells (Fig. 60, B). No matter what the position of the grain may be, the tube always penetrates the tissue of the nucellus adjacent to the chamber. The tube in *Zamia* does not branch before entering the nucellar tissue, and only

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occasionally afterward (Fig. 65, A). During the early development of the tube, the prothallial cells increase in size, becoming broader and longer. The first prothallial cell pushes out into the second, which becomes shaped like a concavo-convex lens, and is crescent-shaped in cross-section (Fig. 60, B, C). As stated in a preceding paragraph, the behavior of the tube nucleus is similar to that in *Cycas*.

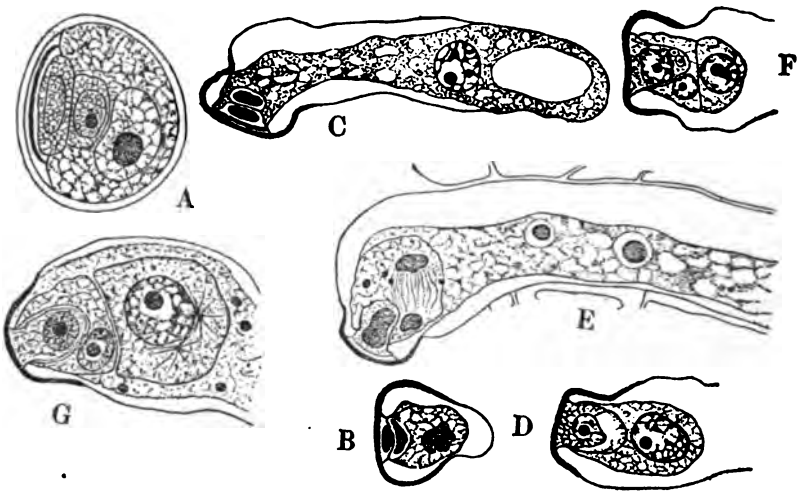

FIG. 60.—Microspore and development of male gametophyte in *Zamia*.—(After Webber.)

A, mature pollen grain; at point of attachment of the two prothallial cells, on left, a dark crescent-shaped line represents a layer in wall of spore, which may be the remains of a third resorbed prothallial cell.

B, germinating pollen grain, early stage. The two prothallial cells have not yet begun to increase in size.

C, later stage of germinating pollen grain; the tube nucleus has increased in size and passed out into tube; prothallial cells unchanged.

D, proximal end of pollen tube; the two prothallial cells have increased in size, the first having crowded out into the second in a marked degree.

E, proximal end of pollen tube; nucleus of second prothallial cell, antheridial cell, in telophase of division, lower end of mitotic figure being crowded to one side by the encroaching first prothallial cell.

F, prothallium in proximal end of tube, after division of antheridial cell into stalk and generative cell.

G, prothallium in later stage of development after appearance of blepharoplasts; the double plasma membrane, separating first prothallial cell and stalk cell, shows that there are two distinct and independent cells of separate origin.

A little later the second cell has arched out very greatly, and the increase in size of the first prothallial cell has brought the second, or antheridial cell, out beyond the limits of the pollen grain and into the tube (Fig. 60, D). However, the prothallium remains in connection with the wall of the pollen spore until the spermatozoids are mature.

The next important step in the development is marked by the division of the second prothallial cell into the stalk cell and generative

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cell (body cell) (Fig. 60, E). In this figure the division is in the telophase, the two daughter-nuclei being still connected by the connecting fibres. Owing to the crescent shape of the cell the spindle lies at an angle to the major axis of the prothallium, the lower nucleus being crowded to one side by the position of the first prothallial cell, while the upper nucleus occupies a central position in the upper half of the cell, which, when the wall is formed, will become the generative cell (body cell, central cell). The lower nucleus becomes the nucleus of the stalk cell. Fig. 60, F, represents the next stage in which the division is complete. A distinct transverse plasma membrane is formed just above the apex of the first prothallial cell which is almost entirely surrounded by the stalk cell. It is clear that should the plasma membrane separating the generative from the stalk cell be very delicate and somewhat obscured, the nucleus of the stalk cell would appear to be forced out to one side. For this reason it seems possible that the plasma membrane separating stalk and generative cells in *Cycas* was overlooked by Ikeno. In *Ginkgo* the first prothallial cell, which according to Webber is also surrounded by the stalk cell, was considered by Hirase ('98) to be strands of cytoplasm in the second prothallial cell. Miyake ('02), who has also examined *Ginkgo*, confirms the observations of Webber.

At the stage of Fig. 60, F, according to Webber, the nucleus of the generative cell is 9.79 μ in diameter, that of the stalk cell 7.12 μ, while the first prothallial cell is 8.9 μ in diameter. The entire prothallium is 29.37 μ long by 16.91 μ wide.

Neither during the division of the second prothallial cell into stalk and generative cell nor for some time afterward was anything observed in the cell in connection with the spindle, or elsewhere, that suggested a young blepharoplast. It is not until the generative cell has increased considerably in size that the first traces of the blepharoplasts were recognized. At first each blepharoplast consists of a small, deeply staining granule, from which several filaments of kinoplasm radiate, following the meshes of the cytoplasmic reticulum (Fig. 60, G). "The central granule (Webber, '01, p. 31) does not seem to be different in substance from the radiations—stains the same and shows no differentiation of structure. In this stage it is only a half micron in diameter or less, and seems to be scarcely more than the point of the crossing of the filaments of kinoplasm. These granules are located in the cytoplasm about halfway between the nucleus and the cell-wall. Two are formed in each central cell at the same time and apparently independently. They are commonly located on the opposite sides of

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the nucleus, but, in a number of cases in this stage and in a still later stage, they have been found nearer together, frequently less than 45° apart."

The first indication of a differentiation in the blepharoplast as it increases in size is seen in the formation of an outer wall or membrane. The generative cell, which has remained nearly spherical, increases in

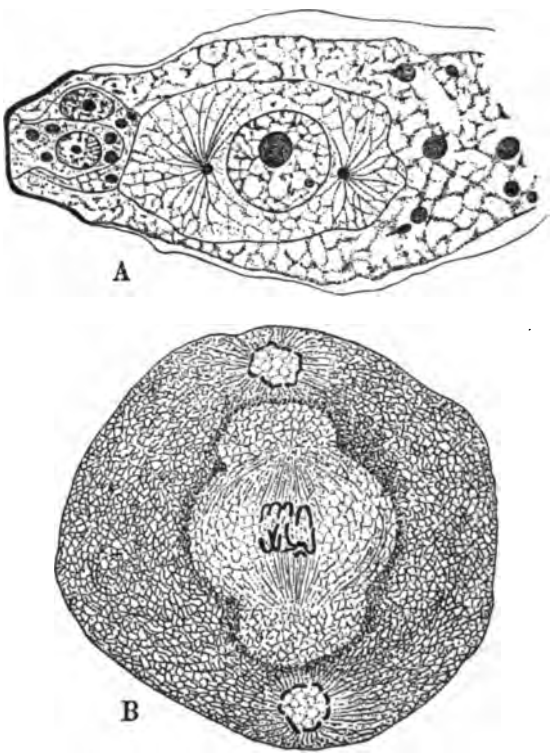

FIG. 61.—Prothallium and a dividing generative cell of *Zamia*.—(After Webber.)

A, prothallium in which generative cell has become large and elongated; the blepharoplasts have taken positions on opposite sides of nucleus, corresponding to longitudinal axis of pollen tube; starch grains have begun to appear in stalk cell.

B, division of generative cell, nucleus in anaphase, showing hyaline cytoplasmic areas around poles; the blepharoplasts, whose outer membranes have separated into pieces or segments, are not connected with spindle.

size and becomes elliptical or oblong, its major axis nearly coinciding with the longitudinal axis of the pollen tube (Fig. 61, A). The blepharoplasts by this time have taken a position on opposite sides of the nucleus on the line of the major axis of the cell. The kinoplasmic radiations are slightly more prominent than the lamellæ or fibrillæ of the cytoplasmic reticulum into which they run and disappear (Fig. 61, A).

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About the first of April the blepharoplasts have reached nearly one-half the size they finally attain. They are more or less vacuolate, and the kinoplasmic radiations, which have become more abundant, extend in many instances quite to the plasma membrane of the cell.

After further growth the generative cell divides into the two cells which develop into the two spermatozoids (Fig. 61, B, and Fig. 62). The blepharoplasts take no part in the division of the nucleus. Although their kinoplasmic radiations become fewer, they do not enter into the formation of the spindle, as the latter develops apparently entirely within the nucleus, and is almost mature before the nuclear membrane has disappeared. In the spindle stage of this division the blepharoplast is seen to have undergone a noticeable change. It has increased in size and its outer membrane has separated from the contents, which are somewhat shrunken. The outer membrane has separated into fragments or plates, and appears now as a broken line (Fig. 61, B). The kinoplasmic radiations have almost disappeared. The reticulum of the cytoplasm about the blepharoplast is so arranged as to suggest radiations. It will be remembered that precisely the same phenomenon occurs in *Cycas*.

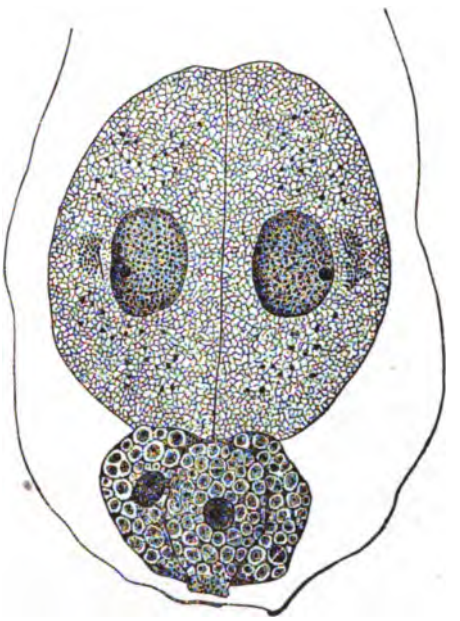

FIG. 62.—Prothallium of *Zamia* in which the generative cell has divided.

The blepharoplasts have separated into granules which are beginning to organize the ciliferous band. The first prothallial cell and stalk cell have become gorged with starch. (The magnification of this figure is only one-half that of A, Fig. 61.)—(After Webber.)

During the anaphase of division the finer structure of the outer membrane, which still consists of a number of segments, is seen to be made up of numerous small granules placed side by side to form the membrane. The central contents, which stained very densely at an earlier stage, have disappeared, giving place to a delicate hyaline reticulum (Fig. 61, B). Webber suggests that the densely staining material which resembled nucleoli in its staining qualities was utilized

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as food material in the growth of the blepharoplasts and other parts of the cell. During the telophase the blepharoplast is represented by a more or less irregular or spherical mass of granules, which have evidently been derived by the breaking up of the membrane. "It would seem that the outer membrane of the blepharoplast breaks up into numerous segments or granules, which assume a roundish or elliptical form, and through the action of the cytoplasm become crowded together in a mass occupying the position of the original blepharoplast."

About the time of the reconstruction of the daughter-nuclei and the formation of the plasma membranes separating the cells, the develop-

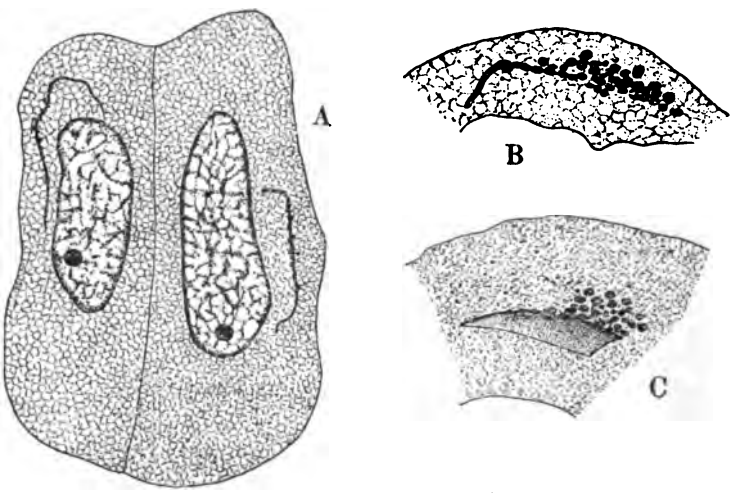

FIG. 63.—Further development of blepharoplast.—(After Webber).

A, two attached spermatozoid mother-cells (spermatids) resulting from division of generative cell; the band of blepharoplast is being formed by fusion of granules.
B, fusion of granules to form the band.
C, formation of ciliferous band by fusion of granules, more highly magnified.

ment of the band, which is to bear the cilia, begins. It appears first as a short, delicate, and deeply staining line extending from the mass of granules toward the nucleus (Fig. 63, A). A little later a similar line or band can be seen on the opposite side of the mass of granules. From Fig. 63, B, it is apparent that the band is developed more or less directly from the granules. The band, which at first is very narrow, increases appreciably in width (Fig. 63, B, C). The further development of the band with its cilia and the transformation of the daughter-cell into a spermatozoid closely resembles that of *Cycas*, already discussed at some length in the preceding pages, with the very noteworthy exception that in *Zamia* there is no nuclear beak formed,

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which is in contact with one end of the blepharoplast in the earlier part of its development (Fig. 63, A).

The mature spermatozoid is also quite similar in structure to that of *Cycas*, consisting of a large nucleus completely surrounded by a layer of cytoplasm in which the ciliferous band, or blepharoplast, is located just beneath the plasma membrane. The blepharoplast is in the form of a helicoid spiral, making about five or six turns counter clock-wise and embracing about one-half of the body of the cell (Fig. 65, B). The spermatozoid, as in the ferns, is a transformation of the entire cell and, therefore, a true spermatozoid.

The development of the spermatozoid in *Ginkgo* according to Hirase ('98) is quite similar to that in *Cycas* as described by Ikeno. In the generative cell of *Ginkgo* Webber ('97) and Hirase ('98) find that, when the nucleus becomes strongly flattened or lenticular, a large nucleolus-like body appears on either side of the nucleus between the nuclear membrane and the young blepharoplasts. Other similar but smaller bodies are sometimes present in the cell. Accompanying these two bodies Hirase finds coarsely granular cytoplasm. The bodies in question react toward stains much as do nucleoli, and, since they disappear at a later stage, it is probable that they represent merely extranuclear nucleolar substance.

Miyake ('02) finds that after the division of the generative cell in *Ginkgo* a cell-wall is formed between the two daughter-cells, and that a distinct and firm wall was always found around the two spermatozoids. The fact that a wall is or is not formed about the daughter-cells, *i. e.*, the mother-cells of the spermatozoids, does not affect the morphological rank of the spermatozoid.

The mature spermatozoid of *Zamia* is probably the largest male gamete known in the plant kingdom, being plainly visible to the unaided eye. When swimming freely and without pressure it is slightly ovate, nearly round or compressed spherical (Fig. 65, B). They vary greatly in size, however, ranging in length from 222 to 332 μ, and in width from 222 to 306 μ.

Ikeno describes the spermatozoid of *Cycas* as being provided with a tail which is merely the elongation of the posterior part of the cytoplasmic mantle. Measured in sections the length was found to be 160 μ and the width 70 μ. The length of the tail was 80 μ or equal to that of the body. Fujii has shown that the tail attributed to the spermatozoid of *Ginkgo* was an artifact, and this statement has been confirmed by Miyake. Since no tail exists in *Zamia*, it is probable that that described for *Cycas* may also have been the result of abnormal conditions.

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THE ARCHEGONIUM.

The development of the archegonium in the *Cycadaceæ* and in *Ginkgo*, which is similar to that of *Pinus*, is too well known to require a detailed description in this place. The manner, however, in which the large central cell is nourished during its growth by the immediately surrounding cells of the prothallium is, if Ikeno's observations be correct, a phenomenon of a rather rare occurrence in the Gymnosperms, and merits some special mention. These surrounding cells, which are separated from the central cell by thick cellulose walls, are of a uniform size, each possessing dense cytoplasm and a large nucleus. Before the archegonium is full grown the nuclei of these cells show a fine and distinct threadwork; but, as this organ approaches maturity, the nuclei, with the exception of the nucleoli, are transformed into homo-

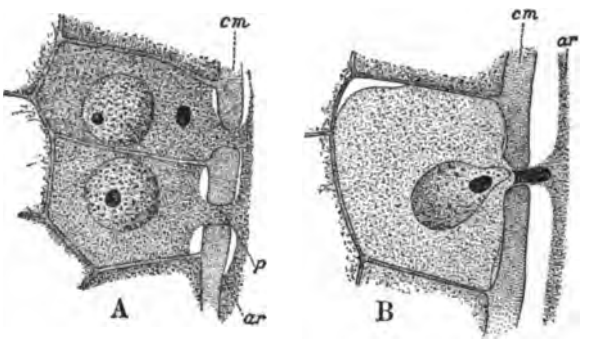

FIG. 64.—Three cells from layer of prothallial cells immediately surrounding upper part of central cell of archegonium of *Cycas*, showing protoplasmic connections between these cells; in B the beak of nucleus extends into plasmic bridge.—(After Ikeno.)

geneous and diffusely staining bodies. This phenomenon is not confined solely to the cells forming the wall of the archegonium, but it may extend to adjacent cells of the prothallium. This nuclear change takes place only in cells near the upper part of the central cell.

Goroschankin has shown that in the *Cycadaceæ* fine cytoplasmic connections exist between the central cell of the archegonium and the surrounding cells. From Ikeno's figures it seems that the cytoplasmic strands in *Cycas* are relatively large, and that large granular plasmic masses pass over bodily into the central cell (Fig. 64, A, B). Frequently the nucleus itself will send out a beak or protuberance toward the nearest plasmic connection. Arnoldi (1900) finds that in several species of *Pinus* and in *Abies* the nuclei from the surrounding cells pass into the egg-cell. The prevalence of condensed nuclei in cells surrounding the upper part of the central cell is explained by Ikeno as

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being due to a greater need of food material by this part of the central cell; for it is here that the greatest activity takes place during the maturing of the egg-cell, which culminates in the formation of the ventral canal-cell. Webber does not find any protoplasmic connections between the egg-cell and those surrounding it in *Zamia*, and so far as the author is aware no such protoplasmic connections exist in the higher Gymnosperms. In *Cycas* the phenomenon described by Ikeno is, if true, probably an adaptation to the rapid transfer of nutritive material from the surrounding cells to the egg-cell.

Strasburger ('01, pp. 550–553), in a late publication on the protoplasmic connections between cells in plants, calls into question the statement that nuclei or nuclear fragments pass bodily through the pits of the surrounding cells into the egg-cell of Gymnosperms as a normal phenomenon, and asserts that it is the result of injury due to pressure or fixing reagents.

There seems to be no doubt that in all Gymnosperms in which the egg-cells reach such an enormous size the cells immediately surrounding the egg contribute directly to the nutrition of the latter, but it is not clear why any of the material should pass over bodily into the egg-cell.

The final step in the development of the archegonium is the formation of the ventral canal-cell, which takes place immediately preceding fecundation, and consequently this cell persists only a short time (Fig. 67, A). It was probably due to this fact that the presence of a ventral canal-cell was not observed by Warming and Treub. Only a plasma membrane and not a cell-wall is formed separating the ventral canal-cell from the egg. It is not at all improbable that in some cases a plasma membrane may not be formed, and such is reported for *Cephalotaxis fortuni* by Arnoldi (1900). The formation of a plasma membrane is, however, of secondary importance in the formation of the ventral canal-cell, for if the nucleus of the central cell of the archegonium divides karyokinetically, and one of the daughter-nuclei becomes the functional egg-nucleus, the division is certainly to be regarded as the formation of a ventral canal-cell whether a plasma membrane is formed or not.

Botanists have sometimes been inclined to refer to the formation of the ventral canal-cell as a maturation process similar to that in the animal egg. Ikeno speaks of this step in the development as the period of maturation (Reifungsperiode), which recalls the formation of the polar bodies in the animal egg, but I do not infer that he considers the two processes homologous. He states, however, that it appears prob-

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able, judging from the karyokinetic figures observed, that the nuclear division leading to the formation of the ventral canal-cell is of the heterotypic type, and takes place essentially as in the first division of the pollen mother-cells of the *Liliaceæ*. This is certainly an error, for in both Gymnosperms and Angiosperms the heterotypic nuclear division occurs in the micro- and macrospore mother-cells and nowhere else in ontogeny. Since the spore mother-cells of the Gymnosperms are homologous with those of the higher plants, we naturally expect to find the heterotypic division in *Cycas* in the first karyokinesis of the macrospore mother-cell. This is made all the more certain by the researches of Juel (1900), who finds in *Larix* that the first nuclear division in the macrospore mother-cell is heterotypic. In *Larix* and

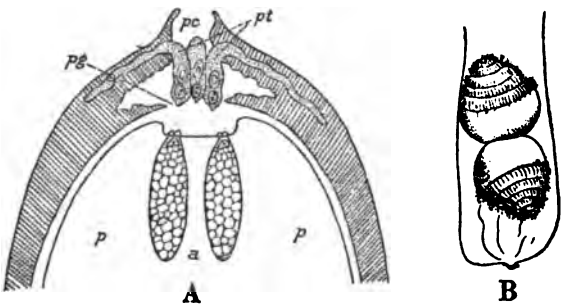

FIG. 65.—Upper end of nucellus; spermatozoids in pollen tube of *Zamia*.—(After Webber).

A, diagrammatic outline of upper end of nucellus, showing proximal ends of pollen tubes growing down into the cavity just above archegonia; *a*, archegonia; *p*, prothallium; *pc*, pollen chamber; *pt*, pollen tubes; *pg*, pollen grain.

B, two mature spermatozoids in proximal end of pollen tube.

in other Gymnosperms the earlier development of the macrospore is precisely the same as in such Angiosperms as *Helleborus*, in which the first nuclear division is heterotypic and homologous with the first division in the pollen mother-cell.

The formation of the ventral canal-cell may represent some sort of a maturation process, and the conclusion that this cell is an aborted egg is tempting, but at our present state of knowledge such an inference is scarcely justifiable.

FECUNDATION.

Soon after its formation the ventral canal-cell disorganizes. The nucleus of the egg passes back gradually toward the middle of the cell, at the same time increasing in size. Finally, when the center of the cell is reached, the nucleus is usually large, being generally longer than broad, and shows the structure of the resting condition.

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During the final stages in the development of the spermatozoid the proximal end of the pollen tube, which is still capped by the exine of the spore, grows downward into the prothallial cavity as in *Zamia* (Fig. 65, A). This cavity in *Cycas*, according to Ikeno, is filled with a watery fluid derived largely from the archegonia, and in which the spermatozoids swim on escaping from the pollen tube. Webber is of the opinion that in *Zamia* this fluid is derived largely from the pollen tube.

The spermatozoids in Cycas, on escaping from the pollen tube, swim about rapidly, and in a short time penetrate the egg. That part of the egg at which a spermatozoid enters is depressed, giving the impression that it came against the egg with some force. The nucleus of the spermatozoid now escapes from its cytoplasmic mantle and migrates toward the nucleus of the egg. The cytoplasm and blepharoplast are left in the upper part of the egg as in *Zamia* (Fig. 66, A, B), where they undergo disorganization. It frequently happens that several spermatozoids reach the egg, but, as a rule, only one penetrates into its interior, the others remaining at the surface. Whether more than one male nucleus ever fuses with the egg-nucleus is not known.

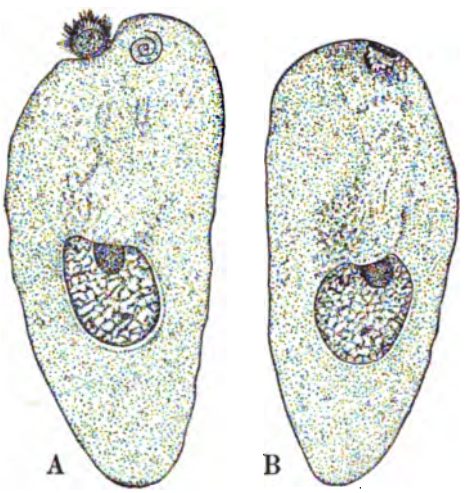

FIG. 66.—Fecundation of egg-cells in *Zamia*.—(After Webber.)

A, egg-cell immediately after coming together of male and female nuclei; the ciliferous band of fecundating spermatozoid lies in upper end of egg; a second spermatozoid trying to gain entrance is shown at apex of egg.

B, similar to A, but showing longitudinal section of ciliferous band in upper end of egg.

When male and female nuclei come in contact they are readily distinguished from each other, the male being smaller, with a more finely granular threadwork. Both are in the resting stage. The male nucleus seems to press against the female, forming a depression in the latter. In a short time the male nucleus is completely imbedded within the egg-nucleus; the membrane of the male nucleus disappears, and the two nuclei fuse so completely that the fusion nucleus can scarcely be distinguished from an unfecundated nucleus of the egg.

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The processes incident to and accompanying fecundation in *Zamia* differ only in minor details from those of *Cycas*. Certain phases of these processes, however, as observed by Webber ('97, I, II, III), are of special interest and importance. They are described as follows ('97, II, p. 18):

The proximal ends of the pollen tubes . . . which grow downward through the apical tissue of the nucellus into a cavity formed in the prothallium above the archegonium, have increased in length until the ends almost or quite touch the neck cells of the archegonia, which protrude into the same cavity (Fig. 65, A). It is interesting to note that the pollen tubes when they enter the prothallium cavity, which is filled with air, do not grow at random, but bend slightly outward and grow directly toward the archegonia. . . . The protruding tip formed by the old pollen grain is plainly visible with a hand lens, and is evidently the point which first comes into contact with the neck cells of the archegonia. The neck cells are also distended and turgid, and are evidently easily broken. If in this stage the end of a pollen tube be touched very lightly with the flat side of a scalpel it bursts, and the antherozoids, together with a drop of the watery contents of the pollen tube are quickly forced out, and the pollen tube immediately shrivels up into a shapeless mass. . . . The pollen tube evidently grows down until the end is forced against the neck cells, when the tube bursts, discharging the mature antherozoids and the watery contents of the tube which supplies a drop of fluid in which the antherozoids can swim. . . . ('97, III, p. 226).

As explained in my previous papers, several antherozoids commonly enter each archegonium, two being usually found and sometimes three or four. The entire antherozoid enters unchanged, swimming in between the ruptured neck cells. Only one of the antherozoids is concerned in fecundation, and the others are usually found between the protoplasm and the wall of the archegonium, presenting their original form and appearance, or in some stage of disintegration (Fig. 66, A). Occasionally one of the antherozoids not concerned in fecundation pushes for a short distance into the contents of the archegonium, as it is always found in such cases to form a distinct body which stains very differently . . . ('01, p. 65). That one which is utilized in fecundation swims into the protoplasm of the archegonium for a short distance, where it comes to rest and undergoes change. The nucleus slips out of its cytoplasmic sheath and passes on alone from this point to the egg-nucleus, with which it unites. The spiral ciliferous band remains at the apex of the egg-cell in the place where the nucleus left. In very numerous instances, just after fecundation, it has been discovered in this position, and there can be no doubt that this process is the one normally occurring. It shows very plainly and presents nearly the original form of the spermatozoid (Fig. 66, B), but is always stretched out much more than in the normal spermatozoid. . . .

The method of escape of the nucleus from the body of the spermatozoid can only be conjectured. It would seem, however, that the rapid boring of the apical or spiral end into the egg-cell may cause too great a pressure on the large body of the spermatozoid, resulting in its bursting and freeing the nucleus,

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while the cilia motion continues probably some time longer, carrying the band farther along and freeing the nucleus from any hindrance by it. The apex of the spiral end of the spermatozoid invariably enters the egg-cell first, and in all of the cases observed where the nucleus has just escaped from the spermatozoid it has been found a short distance behind the spiral of the spermatozoid, as if it had been forced out and left behind. The function of the cytoplasm of the spermatozoid is still in considerable doubt, but that it fuses with the cytoplasm of the egg-cell is certain. Shortly after the nucleus has broken out of the spermatozoid cell, the thin layer of dense cytoplasm which surrounded it can be seen in a broken, fragmentary form, still somewhat connected with the spiral band. The cytoplasm of the spermatozoid in this stage is very different from that of the egg-cell, being more densely granular and staining more deeply, so that it is easily distinguished. Later, only a coarse granular substance is found inside the spiral coil of the ciliferous band, and it would seem that this is the cytoplasmic matter from the spermatozoid which has mingled with that of the egg-cell. It should be mentioned that the plasma membrane surrounding the spermatozoid has entirely disappeared, no trace of it being visible. It would seem to have fused with some substance of the egg-cell or to have been absorbed in some way.

The male nucleus, when it has escaped from the spermatozoid and is observed lying in the cytoplasm at the apex of the egg-cell, is a loose, open structure, seeming to have but little kinoplasmic and chromatin matter. The passage to the nucleus is evidently a rapid one, as few stages have been found between the above and the completion of fecundation. In some instances the path over which the nucleus travelled in reaching the egg-nucleus is discernible by the arrangement of the granules in the cytoplasm, showing the direction of the passage.

The egg-nucleus, previous to fecundation, is elliptical and is located slightly below the center of the enormous egg-cell which is about 3 mm. long by 1.5 mm. wide (Fig. 66, A, B). The egg-nucleus is of enormous size, comparatively, being plainly visible to the unaided eye. It is composed of an open, coarse reticulum. So far as the writer has observed there is no depression or "empfängnisshöhle" in the upper part of the nucleus where the sperm-nucleus enters, as was found by Ikeno in *Cycas*. No special attention has been given to this matter, however, and further observation may show it to be present. The male nucleus in entering the egg-nucleus gradually pushes into it as observed by Ikeno in *Cycas*, and finally becomes entirely surrounded by it. Meanwhile it has changed its structure and become densely granular, differing markedly from the egg-nucleus in this particular. . . . After fecundation is apparently completed the male nucleus appears as a small, nearly round body in the upper portion of the egg-nucleus into which it has penetrated (Fig. 66, B).

Further changes in the sexual nuclei were not followed by Webber, and it is not known whether a fusion nucleus is formed in *Zamia* as described by Ikeno for *Cycas*.

Since the publication of his paper on *Cycas*, Ikeno ('01) has observed the formation of the ventral canal-cell, the process of fecundation and

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the first division of the fusion nucleus in *Ginkgo biloba* (Fig. 67, A, B, C, D). These processes agree closely with those in *Cycas*. In *Ginkgo*, however, the male nucleus at the time of fusion is relatively small, being less than one-tenth the size of the female nucleus. As in *Cycas* and *Zamia*, the male nucleus becomes completely imbedded in

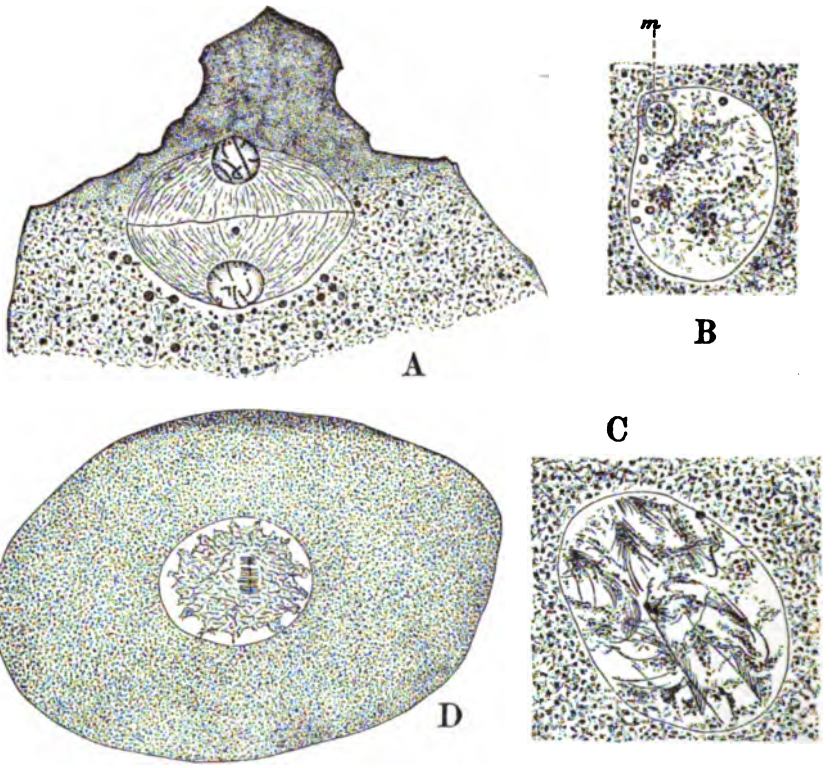

FIG. 67.—Formation of ventral canal-cell, fusion of sexual nuclei, and the division of the fusion nucleus in *Ginkgo*.—(After Ikeno.)

A, apex of central cell of archegonium showing telophase of nuclear division; a cell-plate, or plasma membrane, is formed in the connecting fibers.

B, egg-nucleus into which a male nucleus (*m*) has penetrated.

C, fusion nucleus in prophase of division.

D, fecundated egg-cell showing fusion nucleus in spindle stage of mitosis; the mitotic figure lies within limits of the nucleus whose membrane seems to be still intact.

the female before the dissolution of its membrane. Both nuclei are in the resting condition at the time of fusion.

The spindle of the first karyokinesis following fusion is formed within the nuclear cavity and before its membrane has disappeared (Fig. 67, B, C, D). Nothing is said by Ikeno about being able to distinguish male and female chromatin elements in this division.

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It is interesting to note further that in neither *Cycas*, *Zamia*, nor *Ginkgo* was the stalk or prothallial cell of the pollen tube found in the egg by any of the observers mentioned. These cells are probably disorganized beyond recognition when the contents of the tube are discharged into the egg.

PINUS.

THE MALE AND FEMALE GAMETOPHYTES.

Apart from the absence of motile spermatozoids and the behavior of the male gametophyte, the process of fecundation in the Coniferales, so far as this is well known, is in general similar to that in *Cycas*, *Zamia*, and *Ginkgo*, and it will be necessary only to point out briefly the more important features of difference.

Since the important researches of Strasburger, Goroschankin, and Belajeff upon certain of the higher Gymnosperms, an interesting series of facts has been collected by Dixon ('94), Blackman ('98), Chamberlain ('99), Murrill (1900), Ferguson ('01), and others. The studies of later observers, who used more improved technique, have been confined principally to the genera *Pinus*, *Picea*, and *Tsuga*, and consequently our knowledge of the sexual process in many other Gymnosperms is sadly wanting.

It has been shown by Strasburger ('92) and others that the prothallial cell in the ripe microspore of Pinus and other closely related genera is the last one of a series of two or three cells, and that this cell divides, as in *Cycas* and *Ginkgo*, to form the stalk cell and the generative cell of the antheridium (Fig. 68, A, B). The generative cell (body cell) then divides to produce the two non-motile male gametes, each consisting of a nucleus surrounded by a specially differentiated mass of cytoplasm (Fig. 68, C).

Contrary to *Cycas*, *Zamia*, and *Ginkgo*, the distal end of the male gametophyte, or pollen tube, grows in a more or less direct line from the pollen chamber down through the nucellus to the archegonium, and while the tube seems to be merely a carrier of the gametes, it can and doubtless does act as an absorber of nutriment as well. The probable need of less food by the male gametophyte of the higher gymnosperms may account for the absence of a specially developed absorbing apparatus. This idea is advanced merely as a suggestion and not as an adequate explanation of the difference between the behavior of the tube of *Pinus*, for example, and that of *Cycas* or *Ginkgo*. Other factors may have been more influential during the phylogenetic development of these forms.

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The development of the archegonium is the same as in the lower gymnosperms. The ventral canal-cell is separated from the egg merely by a plasma membrane, which is formed by the connecting fibers, as is usual in the higher plants. It persists for a short time only. In

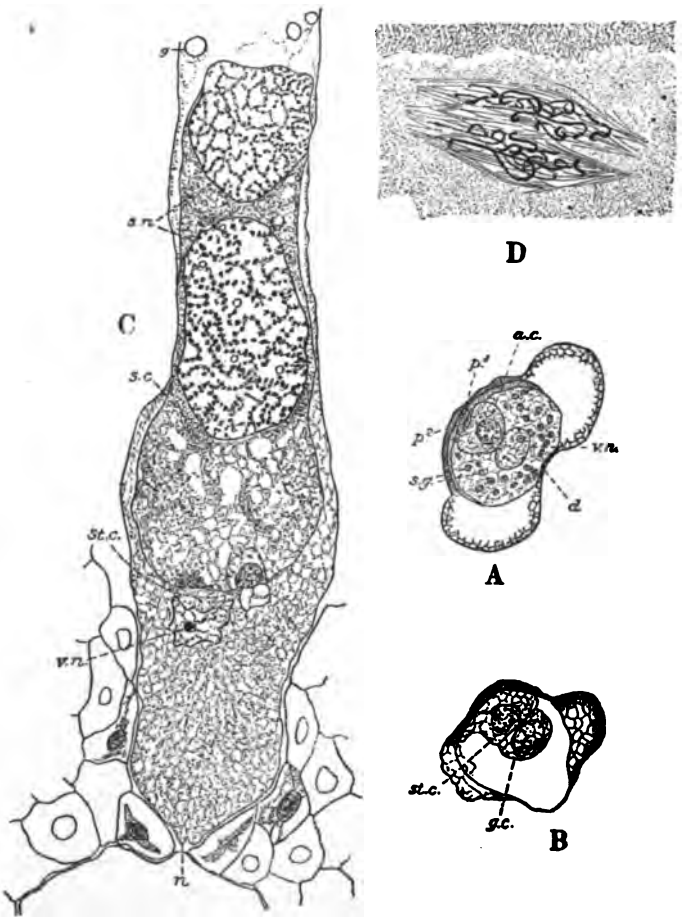

FIG. 68.—Pollen grain, end of pollen tube, and fusion nucleus of *Pinus strobus*.—(After Ferguson.)

A, mature pollen grain. p^1 and p^2, remains of first and second prothallial cells; *a. c.*, antheridial cell.
B, pollen grain in which antheridial cell has divided. *g.c.*, generative cell; *st.c.*, stalk cell.
C, distal end of pollen tube which is pushing between neck-cells of archegonium; the male nuclei (*s.n.*) are of unequal size. *v.n.*, tube nucleus; *st.c.*, stalk cell; *s.c.*, cytoplasm of generative cell.
D, first mitosis following fecundation. The spindle is formed, but the male and female chromatin spirems are still separate and distinct.

Pinus strobus, according to Ferguson, there are probably instances in which the nucleus of this cell is not reconstructed, and this may be true also in other genera and species.

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FECUNDATION.

Goroschanken ('83) observed in *Pinus pumilio* that both male nuclei pass into the egg-cell, and the same fact was established for *Picea vulgaris* by Strasburger ('84). Dixon ('94) seems to have been the first to observe that in *Pinus sylvestris* all four nuclei in the pollen tube, *i. e.*, the two male nuclei, the stalk-cell nucleus, and the tube nucleus pass into the egg-cell of the archegonium. This fact has been confirmed by Blackman ('98) for *Pinus sylvestris*, by Murrill (1900) for *Tsuga canadensis*, and by Ferguson for *Pinus strobus*. According to Blackman the behavior of the four nuclei in *Pinus sylvestris* can be easily followed after their entrance into the egg-cell. The two male nuclei around which the cytoplasm of the generative cell can be no longer observed are distinguished by their larger size. In *P. strobus* one of these nuclei is sometimes larger than the other (Fig. 68, C). The nuclei of the stalk cell and tube are, however, similar, and can scarcely be distinguished from each other.

Within the egg one of the two male nuclei moves toward the nucleus of the egg, the other three nuclei remaining near the upper end of the cell. On its way through the cytoplasm of the egg the functional male nucleus increases in size, and in some cases in substances staining more readily, but in others the increase in size seems to be due to vacuolation. The nucleus of the egg-cell in *Pinus sylvestris* at the time of fecundation presents a strikingly peculiar structure, which differs from that of the female nucleus in all other plants. After the formation of the ventral canal-cell the female nucleus migrates toward the center of the cell, and, by the time it has reached the middle, it has attained an enormous size, and there is developed within it a rather coarse, uniform, and wide-meshed linin reticulum which persists until a later stage (Fig. 69, A). Within this linin reticulum the chromatin is distributed in irregular masses of varying size. These masses may be in the form of irregular lumps as if composed of an aggregate of granules, or in shreds or rods with uneven edges. Sometimes they appear globular as small nucleoli. In fact it is quite difficult to distinguish between some of the small nucleoli and similar chromatin masses, if, indeed, a difference really exists. The quantity of chromatin in the nucleus is proportionally very small. In addition to the linin reticulum there is also present a fine granular substance which appears to be evenly distributed in the nucleus or aggregated along the linin threads. In the former case the nucleus appears more uniformly granular, and its linin reticulum stands out less sharply. The structure of the egg-nucleus in *Pinus sylvestris*, as described by Blackman, agrees with

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that of my own observations, and from the work of Chamberlain ('99) on *Pinus laricio* and Murrill (1900) on *Tsuga canadensis*, it seems that a similarly constructed nucleus is present in these species. In *Pinus strobus* (Ferguson, '01) the structure of the egg-nucleus may vary from a most delicate network bearing minute granules to an interrupted, imperfect reticulum composed of large, irregular, diffusely-

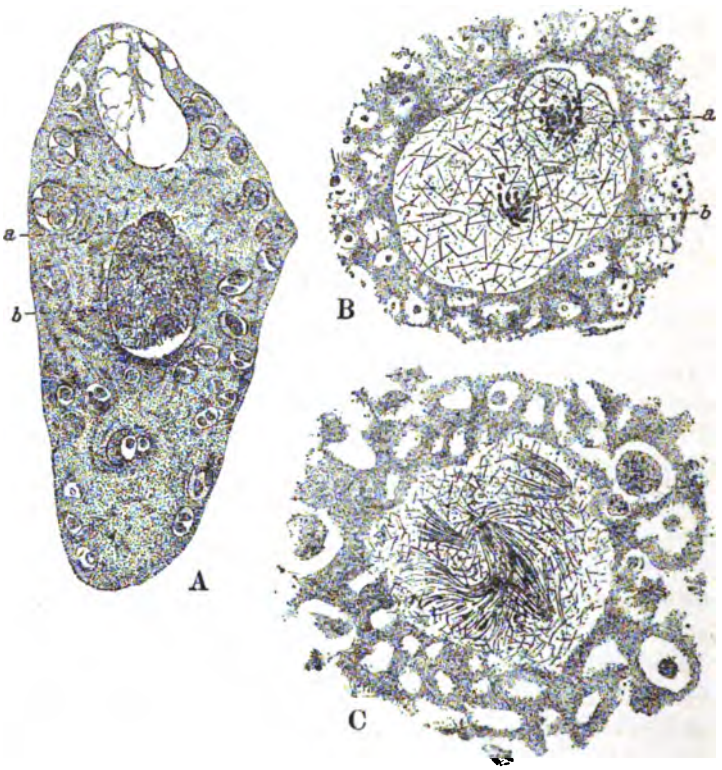

FIG. 69.—The fusion of the sexual nuclei in *Pinus sylvestris*.—(After Blackman.)

A, egg cell showing male nucleus (*a*) entering female nucleus (*b*).
B, later stage in the fusion of the sexual nuclei, parental chromatin masses separate.
C, male and female nuclei fused, multipolar spindle formed.

staining elements. It has one large, vacuolate nucleolus and a variable number of small nucleoli.

The sexual nuclei of *Pinus* on coming together are in the resting condition, and as in *Cycas*, *Ginkgo*, and *Zamia* the male nucleus penetrates bodily into the female nucleus. Here also the male nucleus seems to press with some force against the membrane of the egg-nucleus, thereby forming a concave depression in the latter (Fig. 69, A).

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Although the male nucleus is almost enclosed by the female, actual fusion, according to Blackman, does not take place immediately, since the membrane of the male nucleus is intact (Fig. 69, B). The membrane soon disappears, but the chromatin of the two nuclei does not fuse at this stage and no resting fusion nucleus is formed. With further development the chromatin of each nucleus will give rise to a group of chromosomes, which become arranged upon the spindle of the first division after fecundation where they are seen to be split longitudinally (Fig. 69, C). As has been pointed out for *Ginkgo* (Fig. 67, C, D) the spindle seems to arise entirely within the limits of the female nucleus.

In *Pinus laricio*, according to Chamberlain, after the male nucleus is within the nucleus of the egg, the chromatin of the two pronuclei appear as two distinct masses in the spirem stage. Murrill finds that in *Tsuga canadensis* both nuclei are in the resting condition when actual fusion begins, but he seems to be of the opinion that the identity of the male and female chromatin can be traced until the division of the fusion nucleus, as will be seen from the following:

The chromatin of each nucleus collects in the form of a thick knotted thread near the center of the separating partition, and the two masses remain distinct until the spirem bands begin to segment. Just before the spirems are formed the separating membranes disappear and the nuclear cavities become united. The spindle then arises in a multipolar fashion between and among the two masses, twelve chromosomes being supplied from the chromatin of the sperm and twelve from that of the egg, as described by Blackman for *Pinus sylvestris*.

Ferguson finds in *Pinus strobus* that the two sexual nuclei do not fuse in the resting stage. The male nucleus imbeds itself in the egg-nucleus but does not penetrate its membrane. In each nucleus is developed a chromatin spirem and an achromatic reticulum. The nuclear membranes now disappear, but the two chromatin groups remain distinct until the nuclear-plate stage (Fig. 68, D).

The spindle of the first division following fecundation always lies between the conjugating nuclei and parallel with the outer, free surface of the sperm nucleus. It is multipolar in origin and is probably derived equally from the paternal and maternal nucleus. The spindle fibers appear to arise by a rearrangement of the achromatic nuclear reticula, and are evidently not the expression of a special kinoplasmic substance.

In the stage of the mature spindle of the first division following fecundation in *Pinus austriaca*, the species examined by myself, no distinction whatever could be recognized between male and female chromatin.

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If the results of the several observers referred to in the preceding paragraphs be correct, the behavior of the fusion nucleus in *Pinus* differs not only from that of *Cycas* and *Ginkgo* as described by Ikeno, but also from the fusion nucleus in all other plants, a case described in a species of *Spirogyra* by Chmielewskij excepted.

The fate of the other male nucleus, together with that of the stalk cell and tube, indicates that these structures are consumed as nutrient material. Whether the cytoplasm which is brought into the egg with the male nucleus or as a part of the spermatozoid has any morphological or hereditary value must still remain an open question.

From the standpoint of this work the development and union of the sexual elements in the Gnetales are so imperfectly known that a discussion of the subject will not be given. The process of fecundation in *Gnetum gnemon* has been described in considerable detail by Lotsy ('99), to whose paper the reader is referred.

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CHAPTER VII.—ANGIOSPERMS.

Since the classical researches of De Bary ('49) and Strasburger ('78, '79, '84), especially the latter, the nature of the sexual process in the Angiosperms has been a matter of common knowledge among botanists. It is considered beyond the purpose of this work to discuss the subject historically, and no attempt will be made to present a summary of the various theories that have been advanced from time to time during the past half century upon the homologies of the female gametophyte or embryo-sac. The view held here is that pollen grains and embryo-sacs are respectively micro- and macrospores. The author is of the opinion, as will be seen from what follows, that the preponderance of morphological and cytological evidence indicates clearly that the pollen mother-cell and the embryo-sac mother-cell are undeniably homologous with the micro- and macrospore mother-cells of the archegoniates. The fact that the embryo-sac mother-cell is not provided with a special or well-differentiated cell-wall is almost without significance in determining homologies.

THE EMBRYO-SAC OR FEMALE GAMETOPHYTE.

Although many variations occur among Angiosperms in the development of the embryo-sac, yet in the vast majority of cases this process may be reduced to two forms or types. In the one case a readily distinguishable hypodermal cell of the nucellus, either with or without giving rise to a tapetum, divides into an axial row of four (sometimes three ?) cells, or potential macrospores, the lowermost one developing usually into the embryo-sac. In the second case, which is typified by various species of *Lilium*, the hypodermal cell becomes at once the macrospore. As illustrating these two types respectively, the process of development will be described in *Helleborus fœtidus*, one of the Ranunculaceæ, and *Lilium martagon*.

The macrospore mother-cell of *Helleborus fœtidus* increases greatly in size, becoming much longer than broad in keeping pace with the growth in length of the nucellus. Its nucleus, which lies usually in the upper end of the cell, increases in size simultaneously, as a preparation for the first nuclear division. This period of growth of both cell and nucleus corresponds to the period of growth immediately preceding the first nuclear division in the pollen mother-cell (Fig. 70, A). The nucleus now divides, and, as a rule, there follows a division of

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the cell. The first nuclear division is heterotypic, corresponding in detail with the first karyokinesis in the microspore mother-cell of the same plant. The two resulting cells soon divide again, thus giving rise to the axial row of four cells, the four potential macrospores. The second nuclear division is the same as the second division in the pollen mother-cell. A phenomenon which sometimes occurs in *Helleborus* (and it is probable that it may take place in other plants also) furnishes additional evidence in support of our hypothesis, namely, that the two divisions in this hypodermal cell, or embryo-sac mother-cell, are homologous with the two divisions in the pollen mother-cell. Cell division may not take place until after the second nuclear division, when the four granddaughter nuclei will lie in the upper end of the cell, and the cell-plates are laid down simultaneously (Fig. 70, B). It has been observed also that the four nuclei, instead of lying in one plane as in Fig. 70, B, are sometimes arranged in a tetrad and connected with each other by a system of kinoplasmic connecting fibers, as in the corresponding stage of the pollen mother-cell.

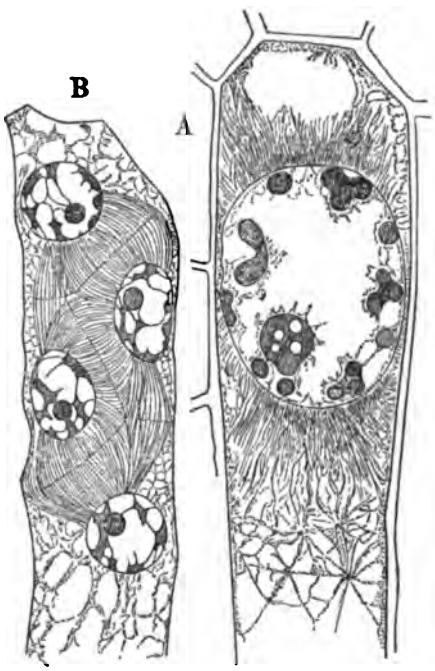

FIG. 70.—Embryo-sac mother-cell of *Helleborus fœtidus*.

A, Upper portion of mother-cell showing nucleus in the prophase of the first mitosis.

B, same less highly magnified, showing the four potential macrospores; in this case cell-division did not follow first mitosis, and the plasma membranes marking out the four cells were formed simultaneously.

The lower cell of the axial row becomes, as a rule, the functional macrospore. It increases rapidly in size at the expense of the other three cells and the adjacent tissue of the nucellus, and develops in the usual way into the embryo-sac.

The unmistakable homology of the macrospore mother-cell of the Angiosperms with that of the Gymnosperms has been very clearly shown by Juel (1900). This author finds in *Larix* that the first and second nuclear divisions in the macrospore mother-cell, which give

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rise to the axial row of four cells, correspond, as in other Gymnosperms, precisely with the first and second divisions in the microspore mother-cell of this plant. In my own opinion the only legitimate conclusion to be drawn from this morphological and cytological evidence is that the macrospore mother-cell of *Larix* is homologous with that of *Helleborus* and other Angiosperms in which the embryo-sac develops similarly.

In the development of the embryo-sac, as typified by *Lilium* and many other monocotyledonous plants, the hypodermal cell does not produce an axial row of four cells, but becomes at once the functional macrospore. With the growth of the nucellus this hypodermal cell increases greatly in size, as does also its nucleus (Fig. 71). The nucleus, after its characteristic period of growth, divides heterotypically. The two resulting daughter-nuclei lie in the ends of the cell. No cell-division follows this nuclear division, although the thickening of the connecting fibers in the equatorial region seems to indicate that a tendency toward cell-division existed (Fig. 72, A). The macrospore continues its growth, and the daughter-nuclei divide. This division is homotypic and corresponds exactly to the second mitosis in the pollen mother-cell. The four resulting nuclei have, as a rule, the orientation shown in Fig. 72, B. Very frequently no vacuole is present at this stage, and the four nuclei are connected with each other and with the plasma membrane by systems of kinoplasmic radiations and connecting fibers. The increase of the cell in length is now rapid, and, as a result, one or more large vacuoles are formed at the center or near the micropylar end of the sac. Two of the four nuclei which are sisters move into the upper, and the other two into the lower end of the cell. In normal cases the nuclei in each end divide so that a group of four nuclei occupies each end. The four nuclei in the micropylar end are arranged either in a plane, or nearly so, or in the form of a tetrad (Fig. 73, A, B). The arrangement and behavior of the nuclei in the chalazal end of the sac is more variable (Mottier, '97).

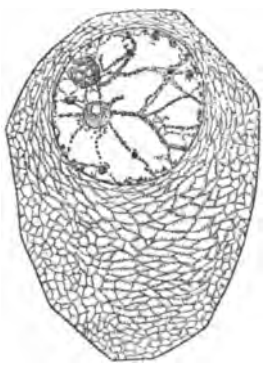

FIG. 71.—Embryo-sac mother-cell of *Lilium martagon* with nucleus showing beginning of prophase of division.

As a rule the two nuclei in the micropylar end of the sac, and it is with these that we are especially concerned, divide simultaneously, and, before cell-plates are laid down, the four resulting nuclei are connected

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by beautiful systems of kinoplasmic connecting fibers. Cell-plates, or plasma membranes, are next formed by the connecting fibers, in a manner common to the higher plants, by which the three cells of the egg-apparatus are differentiated, while a fourth nucleus, the upper polar nucleus and a sister of the egg-nucleus, remains free in the cytoplasm (Fig. 73, B). In A, Fig. 73, three nuclei of the tetrad are shown. The cell-plates are nearly formed, and it is clear that the lower cell to the right will become the egg-cell, while the nucleus to the left is

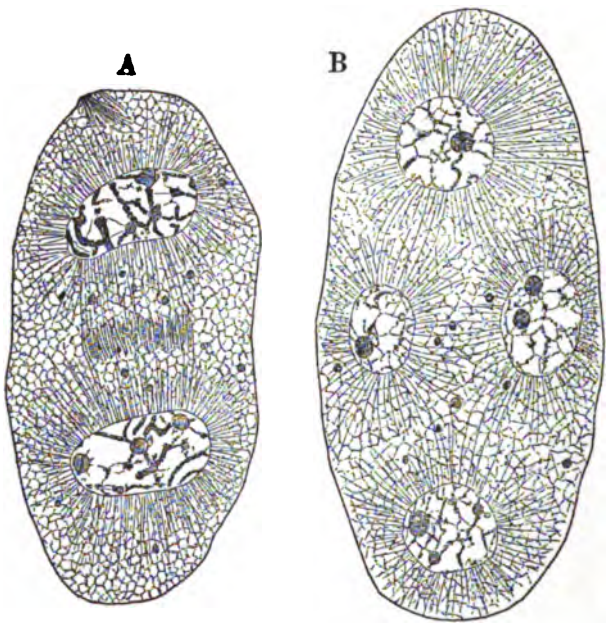

FIG. 72.—Later stages in development of embryo-sac of *Lilium martagon*.

A, the nucleus has divided; the daughter-nuclei are connected by connecting fibers which have shown a tendency to form a cell-plate.

B, close of second nuclear division; the four nuclei are connected with each other and with plasma membrane by kinoplasmic fibers.

unquestionably the upper polar nucleus. The cytoplasm immediately surrounding this nucleus is not delimited by a plasma membrane as in the case of the other three cells. In B, Fig. 73, the relation of all four nuclei is evident.

The antipodal cells in *Lilium martagon* are formed in the same way as those of the egg-apparatus when the process is normal, although the development of these cells is not infrequently variable in this species (Mottier, '97). Among the Angiosperms in general the antipodal cells represent a very variable group both as to number and

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period of duration. In many plants they disorganize immediately after they are formed; in others they may divide repeatedly, giving rise to a larger or smaller mass of tissue which remains functional for a comparatively long time. The development of the antipodal cells into a mass of tissue, whose function is probably concerned with the absorption and elaboration of food materials, may occur in the most widely separated families—a fact which goes to show that this phenomenon is a special adaptation in each specific case and in no way indicative of a closer phylogenetic relationship or a primitive condition.

The typical embryo-sac, or female gametophyte, consists, therefore, of seven cells, one of which, the egg-cell, is the female gamete, while the other cells may be looked upon as vegetative or prothallial cells (Fig. 73, C). The egg-cell may be regarded as the homologue of the egg-cell in the Gymnosperms, and hence a rudimentary archegonium. Whether the synergidæ are to be regarded as rudimentary egg-cells, or merely prothallial-cells, can not be determined at the present state of our knowledge.

As stated in a preceding paragraph, no attempt will be made even to summarize the numerous variations in the development of the embryo-sac that have been observed by the many investigators, since the vast majority of these variations may reasonably be considered as special adaptations, and as such are of small theoretical importance.

One of the many interesting cases about which there is likely to be much diversity of opinion will be briefly mentioned. This is found in the development of the embryo-sac of *Peperomia pellucida*, as described by Campbell ('99, '01) and Johnson (1900). In this species sixteen nuclei are present in the mature embryo-sac. Of these one becomes the nucleus of the egg, one the single synergid, and several, usually eight, fuse to form the endosperm nucleus. The remaining nuclei, according to Johnson, degenerate, but Campbell finds that they are scattered in the sac, each developing about itself a cell-wall much as do the antipodal cells of many Angiosperms. Johnson regards the peculiarities of the embryo-sac in *Peperomia* as secondarily acquired from the typical form, while Campbell looks upon them as primitive, recalling such forms among the Gymnosperms as *Gnetum gnemon* (Lotsy, 1900).

In the development of the embryo-sac, as typified by *Lilium*, the two cell-divisions which result in the axial row of four cells in *Helleborus* are wanting, and the question arises whether the hypodermal cell of *Lilium*, for example, which develops directly into the embryo-sac, is homologous with the hypodermal cell of *Helleborus*, or only

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with that one of the axial row which develops into the embryo-sac. The view held by the author is that the hypodermal cells in both cases are macrospore mother-cells. In *Lilium* this macrospore mother-cell becomes at once the macrospore, while in *Helleborus* it gives rise to four spores. In both cases the reduced number of chromosomes is present, and the egg-cell of *Lilium* is hereditarily the equivalent of the egg-cell in *Helleborus*. The number of cell-divisions elapsing between that period in which the reduced number of chromosomes appears and the differentiation of the sexual cells is of no importance, since in many ferns, for example, thousands of cell-divisions occur between these points in ontogeny. It seems, therefore, that the view held here not only does no violence to either the facts of morphology or cytology, or to the most widely accepted theory concerning the significance of the reduction of the number of chromosomes, but it is also in complete harmony with these facts.

THE MALE GAMETOPHYTE.

As in the case of the embryo-sac, the development of the male gametes in the microspore or in the pollen tube, the male gametophyte, is so well known that only the briefest mention of it is necessary.

In the microspore of *Lilium*, in which the cytological details are probably best understood, the antheridial or generative cell is clearly differentiated from the remaining cytoplasm of the spore by a plasma membrane. The generative cell is moon-shaped or crescentic in *Lilium candidum* and *L. martagon*, and its cytoplasm behaves somewhat differently toward certain stains,[1] so that the contrast between the generative cell and the cytoplasm of the tube cell is often very striking. Strasburger ('98), who attributes a fibrillar structure to the cytoplasm of the generative cell, regards it as kinoplasm, and since some cytoplasm accompanies the male nucleus into the embryo-sac, the theory may not be without significance. In *Lilium* and in many other Angiosperms the generative or antheridial cell divides in the pollen tube to give rise to the two male gametes, but in some instances this division takes place in the spore. Each male gamete consists, therefore, of a nucleus surrounded by a small portion of cytoplasm derived from the generative cell.

Nothing need be added here concerning the growth of the pollen tube toward the egg-cell of the embryo-sac. The result is the same whether the tube enters through the micropyle or chalaza. The end of the tube may enter the sac at one side of one of the synergidæ, in

[1] *E. g.*, Flemming's triple stain.

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which case only one of these cells is at once disorganized, the other retaining its normal structure for some time, or it may enter between the two synergidæ, when both cells are destroyed almost immediately.

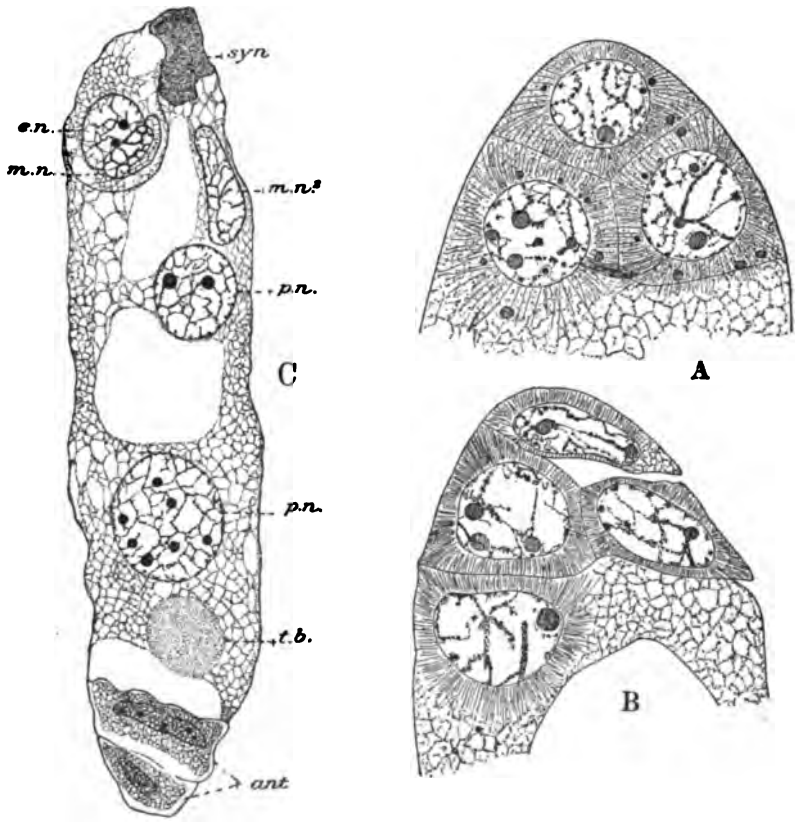

FIG. 73.—Formation of egg-apparatus and mature embryo-sac in *Lilium martagon*.

A, telophase of third mitosis; the four nuclei, three only shown, form a tetrad; the lower nucleus to the right is the egg-nucleus, the one to left the upper polar nucleus; plasma membranes delimiting the three cells of egg-apparatus are just formed.

B, same stage, perhaps a little later, showing all four nuclei in a plane; the lower nucleus on left is the upper polar nucleus.

C, mature embryo-sac into which the male nuclei have been discharged. *e.n.*, egg-nucleus; *m.n.*, male nucleus applied to that of the egg; *m.n.*², second male nucleus approaching upper polar nucleus; *syn.*, disorganized synergid; *p.n.*, polar nuclei; *t.b.*, trophoplasmic body; *ant.*, antipodal cells.

As soon as the end of the pollen tube enters the embryo-sac it opens, discharging the two male gametes and other contents. One of the male nuclei enters the egg-cell and applies itself to the nucleus of the egg, while the other passes on into the cavity of the sac (Fig. 73, C). As soon as the male nuclei have been discharged into the

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embryo-sac and can be distinctly recognized, no trace of the cytoplasm which accompanied them in the tube can be distinguished, so that the exact behavior of this cytoplasm is unknown. Consequently we are concerned here solely with the union of the nuclei.

THE FUSION OF MALE AND EGG-NUCLEI.

We shall follow first the male nucleus which fuses with that of the egg-cell. It is presumably the first male nucleus which escapes from the pollen tube that unites with the nucleus of the egg, but positive proof on this point is wanting. In certain species of *Lilium*, and various observers have shown this to be true of many other Angiosperms, the male nucleus, when observed in the egg-cell, is frequently sausage-shaped, worm-like, or S-shaped (Mottier, '97), making one or more spiral-like turns, which is suggestive of a worm-like motion, but positive proof of any such movement is wanting. It applies itself to the nucleus of the egg, retaining the form mentioned for some time (Fig. 74, A). The structure of the two sexual nuclei at this stage is accurately shown for *Lilium martagon* in this figure. The two nuclei are in the resting condition, although the chromatin of the male nucleus is a little more regularly arranged. The male nuclei when in the embryo-sac stain a deeper red, safranin, gentian violet and orange G being used, than the other nuclei of the sac, and for that reason they may be readily recognized. As fusion progresses, the nuclei become quite alike in shape, size and structure (Fig. 74, B). Their membranes gradually disappear at the place of contact, their cavities become one, and the resulting fusion nucleus, which is in the

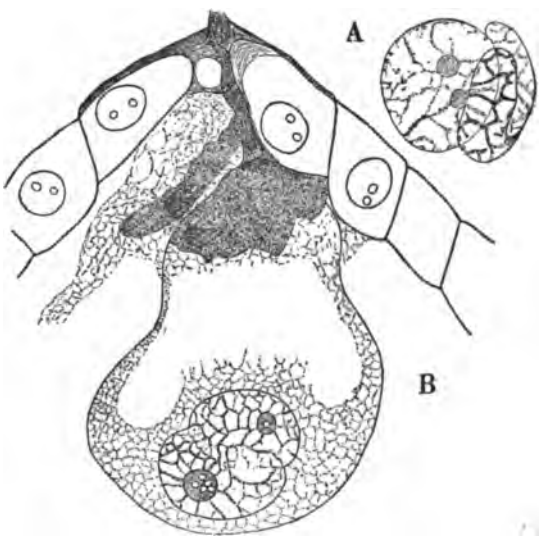

FIG. 74.—Fusion of sexual nuclei.
A, vermiform male nucleus applied to egg-nucleus, *Lilium martagon*. B, egg-cell of *Lilium candidum*, showing sexual nuclei in act of fusing; the nuclear membranes have disappeared at place of contact.

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resting condition, can scarcely be distinguished from the nucleus of an unfecundated egg. The nucleoli finally unite also.

The worm-like or S-shape form of the male nucleus in *Lilium*, first described by the author in 1897 (Mottier, '97, p. 23), has since that time attracted the close attention of students of fecundation generally. Guignard, having observed the same phenomenon in 1899, concluded to designate these vermiform nuclei as antherozoids, evidently attributing to them the power of locomotion. As a matter of fact these nuclei do not possess cilia or any other cytoplasmic organ of locomotion, nor have the male nuclei in any Angiosperm been found to possess any such structures. Nuclei in many vegetative cells of both plants and animals are known to be able to change their form, and the fact that in the embryo-sac the male nuclei may assume a worm-like shape, which merely suggests a squirming or vermiform motion, is not a sufficient reason for designating them as spermatozoids. So far as is known, all spermatozoids are provided with a cytoplasmic organ of locomotion, existing in the form of a cilium or cilia, and it certainly does not conduce to clearness to apply this term to the male nuclei of the Angiosperms. Strasburger (1900) claims that the vermiform nucleus moves passively in the embryo-sac, basing his opinion upon observations of the embryo-sac of *Monotropa* in the living condition. A streaming movement was seen in the cytoplasmic strand connecting the egg-cell with the endosperm nucleus, and, in the light of this fact, it is highly probable that the second male nucleus is carried to the endosperm nucleus by that means.

THE FATE OF THE SECOND MALE NUCLEUS IN THE EMBRYO-SAC.

The fact that one of the male nuclei fuses with a polar nucleus, or with the endosperm nucleus in certain lilies and in species of widely separated families, has also aroused a keen interest among botanists, and has called forth much interesting and suggestive speculation. In 1897 the author called attention to the fact that the second male nucleus in *Lilium martagon* applied itself to one of the polar nuclei, but the actual fusion was not observed. The plants from which the material was obtained produced few or no seeds that year, and all preparations of embryo-sacs, examined at a time when normally fecundated eggs should have been present, gave only evidence of disorganization, and it was concluded that probably a fusion of the nuclei did not proceed further, which under the circumstances may have been true. Later, other investigators as well as the author have observed this nuclear

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fusion in species of *Lilium* (Fig. 75, A, B, C). An account of the fusion of one of the male nuclei with the polar nuclei was first published by Nawaschin ('99) and made known to botanists in general by a reference in the Botanisches Centralblatt.

Guignard ('99) in the same year published the results of his observations confirming the statement of Nawaschin. He figured the second vermiform male nucleus in contact with one or both polar nuclei, but none of Guignard's figures showed an actual fusion. Although we are justified in assuming that sexual nuclei, when brought in contact, will fuse, yet the possibility is not excluded that since the sexual nuclei remain side by side for some time before fusion takes place, the causes which have been long known to operate in preventing the formation

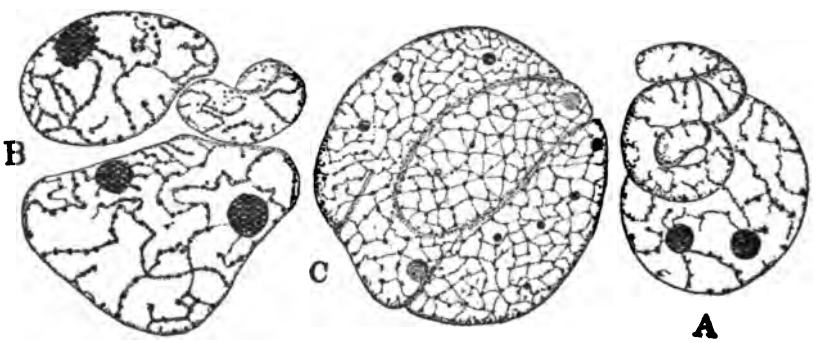

FIG. 75.—Fusion of second male nucleus with polar nuclei in *Lilium martagon*.
A, an S-shaped male nucleus applied to the upper polar nucleus.
B, second male nucleus (shown only in part) and the two polar nuclei close together.
C, all three nuclei fusing.

of seeds in certain species of *Lilium* may also prevent the complete fusion of these nuclei after having come in contact.

The fusion of a male nucleus with the endosperm nucleus has received different interpretations at the hands of the several investigators. Nawaschin (1900), H. De Vries ('99, 1900) and Correns ('99) evidently see in this fusion a true sexual process, basing their conclusion largely upon the hybrid character of the endosperm of certain varieties of *Zea mays*. Guignard in his paper upon *Tulipa celliana* and *T. sylvestris* regards the process as a pseudo-fecundation.

From a series of important experiments on the hybridization of several varieties of *Zea mays*, Webber (1900) arrives independently at the same conclusion as De Vries, namely, that certain phenomena of xenia are the result of the fusion of one of the male nuclei with the endosperm nucleus. As a result of the crossing, the endosperm, produced in the same embryo-sac with the hybrid embryo sporophyte,

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shows certain well-marked characters of the male parent, and according to the hypothesis of Webber, De Vries, and others, these hybrid characters are transmitted by the male nucleus. In some cases the endosperm does not reveal hybrid characters, but only those of the mother plant, and Webber explains the fact by assuming that in those cases the endosperm nucleus may not have been fecundated. As an explanation of another peculiar feature of xenia in certain varieties of maize, which is shown by a variegated or mosaic endosperm, Webber suggests that probably the second male nucleus may not have united with the endosperm nucleus, but it may have been able to divide independently. If this should occur, there would then be formed in the embryo-sac nuclei of two distinct characters, one group from the division of the endosperm nucleus and one from the sperm nucleus. Or a second hypothesis lies in the probability that the second male nucleus fuses with one of the polar nuclei, and that after fusion the other polar nucleus is repelled and develops independently. In view of the fact that in the sea-urchin (Boveri, '95) the male nucleus is capable of independent division under certain circumstances, these hypotheses are certainly very suggestive, but they have, as yet, among plants no support based upon observation, especially since parthenogenesis is unknown in maize. Before these suggestions can be of much value in explaining the phenomenon, it is necessary to know whether a male nucleus is of itself capable of division in the embryo-sac, and whether one of the polar nuclei without having united with the other or with a sperm nucleus is also capable of independent division.

Although the union of a male nucleus with the endosperm nucleus may be conclusively shown to be the cause of hybrid endosperm in maize, yet that fact alone is not sufficient to justify the unqualified conclusion that the fusion represents a real fecundation. Strasburger, in discussing this question at some length in the Botanische Zeitung (pp. 293–316, 1900), argues forcibly against the doctrine of a double sexual process as understood by Nawaschin, and proposes a different interpretation of the two sets of nuclear fusions. For the union of the male nucleus and that of the egg-cell which results in an individual sporophyte, the expression *generative fecundation* is used, while the fusion of the other male nucleus with the endosperm nucleus is designated *vegetative fecundation*. In the interpretation of Strasburger, the need of generative fecundation by means of sexual nuclei of different origin lies in the equalization of individual variations, which is necessary for the continuance of the species, while in vegetative fecundation there is merely the

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manifestation of a growth stimulus. Vegetative fecundation according to this interpretation finds its parallel in such phenomena as described by Klebs ('98, 1900), Loeb ('99, '01) and Nathansohn (1900), in which, by means of physical or chemical stimuli, such as increased temperature or an increase of the osmotic power of the surrounding fluid, unfecundated egg-cells have been made to develop parthenogenetically through certain embryonic stages. According to the view of Strasburger, therefore, sexual reproduction embraces fundamentally two great and far-reaching factors, namely, the union of hereditary elements and the imparting of a growth stimulus. In the fusion of a male nucleus with the endosperm nucleus, only one of these factors, the stimulus to growth, is manifested, since the interrupted growth of the endosperm is enabled to continue. The result is the same whether the second sperm nucleus unites with the endosperm nucleus or not, and furthermore because the endosperm is not an individual in the sense that the embryo sporophyte is an individual. It is further true that the endosperm nucleus may divide and give rise to several nuclei before the contents of the pollen tube are discharged into the embryo-sac, and in case that no pollen tube reaches the embryo-sac, these same endosperm nuclei never continue their development. It is reasonable to conclude, therefore, that a growth stimulus may be imparted to the endosperm by the act of fecundation in the egg-cell, just as the vegetative tissue of certain parts of the pistil are stimulated to growth by the presence of the pollen tube.

Many who agree with Strasburger may probably not consider it necessary or advisable to use the term "vegetative fecundation." The author does not see the necessity of associating the idea of fecundation with this process of nuclear fusion, for the reason that nuclear fusions in vegetative cells do not signify an act of fecundation. In the light of all the known facts, it seems that we have to do here with purely vegetative fusions, and that we are not justified in attributing to such nuclear fusions the idea of sexuality. Although the upper polar nucleus is the sister of the egg-nucleus, it does not necessarily follow that the former is also a female nucleus, since it is certainly not true that the sister cells of egg-cells are even potential gametes. If such an assumption were accepted, then the ventral canal-cell of the archegoniates might be considered an egg-cell, a doctrine to which the author can not, as yet, subscribe.

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